

## METHODS AND REAGENTS FOR THE DETECTION OF MELANOMA

## BACKGROUND OF THE INVENTION

Cutaneous malignant melanoma is a common, aggressive cancer with growing incidence. It is a serious healthcare problem with over 55,100 new cases anticipated in 2004 in US, and a mortality rate of about 14.5%. Cancer Facts and Figures 2003. American Cancer Society, 2003. The incidence of melanoma continues to rise faster than that of any other malignancy. De Braud et al. (2003). While prognosis of early local melanoma is favorable with 5-year overall survival over 90%, regional lymph node involvement decreases the overall survival rate to 10-46%. Balch et al. (2001). Therefore regional lymph node (LN) status becomes the most significant prognostic factor in a melanoma patient's survival. Introduction of the sentinel lymph nodes (SLN) technique (Morton (1992)) has increased the sensitivity of melanoma micrometastasis detection compared to H&E staining alone. Yu et al. (1999); and Messina et al. (1999). Nevertheless, even when enhanced by IHC, histological analysis is limited by the ability of light microscopy to recognize the tumor cells. Reverse transcription-polymerase chain reaction (RT-PCR) analysis has recently been proposed for a more sensitive detection of melanoma cells in LN. Many studies, when using well-characterized melanocyte specific markers, such as tyrosinase and MART-I, have demonstrated the presence of these gene transcripts in LNs otherwise found to be negative by routine histology and IHC. Shivers et al. (1998); and Kuo et al. (2003). However, these genes are not specific to tumor cells and cannot be used to discriminate between benign and malignant tissue. In fact, they caused false-positive results in the presence of benign capsular nevi. Takeuchi et al. (2004); Starz et al. (2003); and Gutzmer et al. (2002). Considering that benign nevi are not rare events in the melanoma SLN, the current RT-PCR assays are not useful clinically for diagnostic of melanoma micrometastasis. A recent study, proposed a multi-marker panel, including cancer specific markers for RT-PCR assay in order to increase assay specificity. Hoon et al. (2004). Identification of novel melanoma specific markers remains one of the key questions of melanoma research.

Certain proteins have been shown to be associated with melanoma and its metastases. These proteins or their activities have been used in IHC to identify

metastases and include LICAM (Thies et al. (2002); Fogel et al. (2003)); and S-100 (Diego et al. (2003)).

Nucleic acid tests have been proposed to increase the sensitivity of detection of metastatic melanoma. US Patent Publication Nos. 2002/01 10820; and 2003/0232356.

5 Studies have used markers that include MAGE3, tyrosinase, MART- 1, MITF-M or IL-I, R1, endothelin-2, ephrin-A5, IGF Binding protein 7, HLA-A0202 heavy chain, Activin A ( $\beta$ A subunit), TNF RII, SPC4, CNTF Ra, or gp100 (HMB45) genes. Bostick et al. (1999); Hoon et al. (2001); Palmieri et al. (2001); Wrightson et al. (2001); Gutzmer et al. (2002); Davids et al. (2003); Starz et al. (2003); Rimboldi et al. (2003); Cook et al (2003); Reintgen et al. (2004); US Patent Publication Nos. 2002/0098535; 2003/0049701; US Patent Nos. 5,512,437; 5,512,444; 5,612,201; 5,759,783; 5,844,075; 6,025,474; 6,057,105; 6,235,525; 6,291,430; 6,338,947; 6,369,211; 6,426,217; 6,475,727; 6,500,919; 6,527,560; 6,599,699; WO 96/29430. Where determined, these markers have not been found adequate for sole use in  
10 (2003); Cook et al (2003); Reintgen et al. (2004); US Patent Publication Nos. 2002/0098535; 2003/0049701; US Patent Nos. 5,512,437; 5,512,444; 5,612,201; 5,759,783; 5,844,075; 6,025,474; 6,057,105; 6,235,525; 6,291,430; 6,338,947; 6,369,211; 6,426,217; 6,475,727; 6,500,919; 6,527,560; 6,599,699; WO 96/29430. Where determined, these markers have not been found adequate for sole use in  
15 melanoma diagnosis. Riccioni et al. (2002); Gutzmer et al. (2002); Davids et al. (2003); Goydos et al. (2003); and Prichard et al. (2003).

A number of these markers have also been shown to be indicative of other neoplasias such as ME20M (GP100) for clear cell sarcoma, biliary tract carcinoma and gastric carcinoma. Hiraga et al. (1997); Okada et al. (2001); Okami et al. (2001);  
20 Antonescu et al. (2002); Segal et al. (2003). MAGE3 is also indicative of a number of neoplasias including breast, hepatocellular, renal, neural, lung and esophageal. Yamanaka et al. (1999); Ooka et al. (2000); Suzuki et al. (2000); Cheung et al. (2001); and Weiser et al. (2001). Several melanoma antigen-encoding genes are also expressed in lung cancer. Yoshimatsu et al. (1998).

25 These markers proved to be sensitive but non-specific since they showed positive expression in other cancers and benign melanocytes. Additionally, tyrosinase is expressed in Schwann cells which are present in normal lymph nodes. The lack of specificity alone calls for the development of assays with new or additional markers. H&E histology and IHC remain the "gold standard for the identification of melanoma  
30 and nevus cells in SLNs." Starz et al. (2003). Detection issues in the intra-operative setting make this need even more acute.

Lymph node involvement is the strongest prognostic factor in many solid tumors, and detection of lymph node micrometastases is of great interest to pathologists and surgeons. Current lymph node evaluation involves microscopic examination of H&E-stained tissue sections and IHC and suffers from three major limitations: (a) small foci of cells, are easily missed; (b) the result is not rapidly available, meaning that any positive result in a SLN procedure requires a second surgery for removal of axillary lymph nodes; and (c) only one or two tissue sections are studied, and thus the vast majority of each node is left unexamined. Serial sectioning can help overcome sampling error, and IHC can help identify small foci of cells; this combination, however, is costly and time consuming for routine analysis.

Surgical decisions of regional lymph node dissection can be based on intra-operative frozen section analysis of lymph nodes; however, the sensitivity of these methods is relatively poor, ranging from 50-70% relative to standard H&E pathology, leading to a high rate of second surgeries. Thus, pathologists are not routinely performing intra-operative frozen section analysis or touch print cytology analysis for melanoma patients. Improvements in the sensitivity and specificity of intra-operative assays for melanoma would significantly benefit oncology.

High-density microarrays have been applied to simultaneously monitor expression, in biological samples, of thousands of genes. Studies have resulted in the identification of genes differentially expressed in benign and malignant lesions, as well as genes that might be of prognostic value. Luo et al. (2001); and Wang et al. (2004). Gene expression profiling of malignant melanoma has been accomplished using a microarray containing probes for 8,150 cDNAs. Bittner et al. (2000). These researchers identified several genes that might be associated with aggressive tumor behavior. In recent work, comparison of gene expression profiles of a few melanoma and normal melanocyte cell lines led to the identification of differentially expressed genes and pathways modulated in melanoma. Takeuchi et al. (2004).

#### SUMMARY OF THE INVENTION

Gene expression profiling of an extensive set of clinically relevant tissue samples is provided in the present invention. Total RNA from forty-five primary malignant melanomas, 18 benign skin nevi and 7 normal skin tissues were hybridized on an

Affymetrix Hu1 33A microarray containing 22,000 probe sets. Differentially expressed genes in malignant melanoma as compared to benign tissue were identified. Pathway analysis of the differentially expressed genes revealed an over-representation of genes associated with neural tissue development and activation of amyloid processing signaling pathway. A one-step quantitative RT-PCR assay was used to test a combination of two melanoma specific genes, PLAB and LICAM in a panel of clinically relevant samples that included primary malignant melanoma, benign nevi, melanoma LN metastasis and melanoma-free lymph node samples.

The present invention provides a method of identifying a melanoma by obtaining a tissue sample; and assaying and measuring the expression levels in the sample of genes encoding mRNA corresponding to prostate differentiation factor (PLAB, MIC1) (SEQ ID NO: 1) and L1 cell adhesion molecule (LICAM) (SEQ ID NO: 2); or PLAB, LICAM and neurotrophic tyrosine kinase receptor, type 3 (NTRK3) (SEQ ID NO: 3) where the gene expression levels above pre-determined cut-off levels are indicative of the presence of a melanoma in the sample. The invention further provides a method of identifying a melanoma by obtaining a tissue sample; and assaying and measuring the expression levels in the sample of genes encoding mRNA recognized by the primer/probe sets SEQ ID NOs: 4-6 or SEQ ID NOs: 7-9 and SEQ ID NOs: 10-12 or SEQ ID NOs: 13-15; or SEQ ID NOs: 4-6 or SEQ ID NOs: 7-9 and SEQ ID NOs: 10-12 or SEQ ID NOs: 13-15 and SEQ ID NOs: 16-18 where the gene expression levels above pre-determined cut-off levels are indicative of the presence of a melanoma in the sample.

The invention also provides a method of distinguishing a malignant melanocyte from a benign melanocyte by obtaining a tissue sample; and assaying and measuring the expression levels in the sample of genes encoding PLAB and LICAM; or PLAB, LICAM and NTRK3 where the gene expression levels above pre-determined cut-off levels are indicative of the presence of a melanoma in the sample.

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5 The invention further provides a method of determining patient treatment protocol by obtaining a tissue sample from the patient; and assaying and measuring the expression levels in the sample of genes encoding PLAB and LICAM; or PLAB, LICAM and NTRK3 where the gene expression levels above pre-determined cut-off levels are indicative of the presence of a melanoma in the sample.

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15 cut-off levels are indicative of the presence of a melanoma in the sample.

The invention further provides additional Marker and control genes, the expression of which aid in the claimed methods. These additional genes include up-regulated SEQ ID NOs: 29-467 and down-regulated SEQ ID NOs: 468-978.

20 The primary Marker can be PLAB and is defined herein as the gene encoding any variant, allele etc. including SEQ ID NO: 1. PLAB is also described by Paralkar et al. (1998) and represented by Accession No. AF003934. PLAB is also defined as the gene encoding mRNA recognized by the primer/probe sets SEQ ID NOs: 4-9.

25 The secondary Marker can be LICAM and is defined herein as the gene encoding any variant, allele etc. including SEQ ID NO: 2. LICAM is also described by Haspel et al (2003); and US Patent No. 6,107,476 and is represented by Accession No. NM\_000425. LICAM is also defined as the gene encoding mRNA recognized by the primer/probe sets SEQ ID NOs: 10-15.

30 The invention further provides a kit for conducting an assay to determine the presence of melanoma in a cell sample comprising: nucleic acid amplification and detection reagents.

The invention further provides primer/probe sets for amplification and detection of PCR products obtained in the inventive methods. These sets include the following:

- SEQ ID NO:4 (PLAB forward primer) ggcagaatcttcgtccgca  
 SEQ ID NO:5 (PLAB reverse primer) ggacagtgggtccccgttg  
 5 SEQ ID NO:6 (PLAB probe) cccagctggagttgcacttgcggcc  
 SEQ ID NO:7 (PLAB upper primer) gaacaccgacctcgtccc  
 SEQ ID NO:8 (PLAB lower primer) ggcggcccagagata  
 SEQ ID NO:9 (PLAB probe) cgccagaagtgcggctgggatt  
 SEQ ID NO:10 (LICAM forward) gctgggactgggaacagaact  
 10 SEQ ID NO:11 (LICAM Reverse) ggagcagagatggcaaagaaa  
 SEQ ID NO:12 (LICAM probe) ttccccaccatctgctgt  
 SEQ ID NO:13 (LICAM upper) ccacagatgacatcagcctcaa  
 SEQ ID NO:14 (LICAM lower) ggtcacaccagctcttcctt  
 SEQ ID NO:15 (LICAM probe) tggcaagcccgaagtgcagttcctt  
 15 SEQ ID NO:16 (NTRK3 primer) gccccggcacccttta  
 SEQ ID NO:17 (NTRK3 primer) aacctgccagtgtggat  
 SEQ ID NO:18 (NTRK3 probe) cagatgggtgttttc  
 SEQ ID NO:19 (Tyr upper) actcagcccagcatcattcttc  
 SEQ ID NO:20 (Tyr lower) atggctgtgtactcctccaatc  
 20 SEQ ID NO:21 (Tyr probe) cttctcctcttggcagattgtctgtagctt  
 SEQ ID NO:22 (PBGD upper) ccacacacagcctactttccaa  
 SEQ ID NO:23 (PBGD lower) taccacgcgaatcacttca  
 SEQ ID NO:24 (PBGD probe) aacggcaatgcggctgcaacggcggaatt

- The invention further provides amplicons obtained by PCR methods utilized in the  
 25 inventive methods. These amplicons include the following:  
 SEQ ID NO:25 (PLAB Amplicon)  
 gaacaccgacctcgtccggccccctgcagtcggatactcacgccagaagtgcggctgggatccggcgccacctgca  
 cctgcgtatctctcgggcccgc  
 SEQ ID NO:26 (LICAM Amplicon)  
 30 ccacagatgacatcagcctcaagtgtgaggccagtggcaagcccgaagtgcagttccgctggacgagggatgtgtcca  
 cttcaaaccaaggaagagctgggtgtgacc  
 SEQ ID NO:27 (tyrosinase Amplicon)  
 actcagcccagcatcattctctcctcttggcagattgtctgtagccgattggaggagtacaacagccat

SEQ ID NO:28 (PBGD Amplicon)

ccacacacagcctactttccaagcggagccatgtctgtaacggcaatgcggctgcaacggcggaagaaaacagcccaa  
agatgagagtgattcgcgtgggta

Other genes described herein include up-regulated Markers (SEQ ID NOs: 29-467), down-regulated Markers (SEQ ID NOs: 468-978), PBGD (SEQ ID NO: 979), MART1 (SEQ ID NO: 980), ME20M (GPI00; SEQ ID NO: 981) and MAGE-3 (SEQ ID NO: 982) and various primers and probes (SEQ ID NOs: 983-1011) used in detecting their expression.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1. Flowchart of data analysis.

Fig. 2. Hierarchical clustering on the 15,795 genes that have at least two "present" calls in all samples. Each column is a sample and each row is a gene. Red is up-regulation and green is down-regulation. Purple: melanoma samples; yellow: benign nevi; and blue: normal skin.

Fig. 3. Microarray expression (A) and real time RT-PCR validation data (B) of the selected genes. First fourteen samples from the left are the melanoma tissue samples (red); next seven are benign nevi samples (yellow) and last five are normal skin (blue). For microarray plots x-axis shows intensity values; for PCR plots, x-axis is  $2^{\Delta Ct}$ , where  $\Delta Ct$  is  $Ct$  (Target Gene) -  $Ct$  PBGD.

Fig. 4. Amyloid processing pathway. Adopted from Ingenuity™ Pathway Analysis Software Application. Genes up-regulated in melanoma are red and down-regulated in melanoma are green. Each gene symbol is followed by the fold-change of expression level between melanoma and benign/normal samples.

Fig. 5. One-step quantitative RT-PCR assay of PLAB and LICAM (A) and conventional melanoma markers, gp100, tyrosinase (SEQ ID NO: 999) and MART1 (B). For each plot x-axis represents score for the new markers or the conventional markers. Median scores for each samples category are labeled. Two cut-off levels based on normal (green) and benign (red) samples are labeled on each plot.

## DETAILED DESCRIPTION

The present invention provides methods of qualitatively and quantitatively identifying a melanoma; distinguishing a malignant melanocyte from a benign melanocyte; diagnosing melanocytic lesions with uncertain pathological features; and determining a melanoma patient treatment protocol. The methods further provide aids in patient prognosis, patient monitoring and drug development. The methods rely on assaying and measuring expression levels of various Marker genes encoding mRNAs provided herein where gene expression over a pre-determined cut-off level is indicative of the presence of a malignant melanocyte in the sample assayed.

Cutaneous melanoma is a common, aggressive cancer with growing incidence. Identification of melanoma-specific deregulated genes could provide molecular markers for LN staging assays and further insight on melanoma tumorigenesis. Total RNA isolated from 45 primary melanoma, 18 benign skin nevi, and 7 normal skin tissue specimens were analyzed on an Affymetrix U133A microarray containing 22,000 probe sets. Hierarchical clustering revealed a distinct separation of the melanoma samples from benign and normal specimens. Novel genes associated with malignant melanoma were identified. Differential gene expression of two melanoma specific genes, PLAB and LICAM, were tested by a one-step quantitative RT-PCR assay on primary malignant melanoma, benign nevi and normal skin samples and also on malignant melanoma LN metastasis and melanoma-free lymph nodes. The performance of the markers was compared to conventional melanoma markers such as tyrosinase, gp100, and MART1. The results demonstrated the ability of using a combination of PLAB and LICAM in a RT-PCR assay to differentiate clinically relevant tissue samples containing benign or malignant melanocytes.

High-density cDNA and oligonucleotide microarrays allow simultaneous monitoring of the expression of thousands of genes. Microarray technology provides a quantitative measurement of mRNA abundance and has gained acceptance as a tool for marker discovery based on gene expression. In the context of cancer research, microarray analysis has identified genes differentially expressed in benign and malignant lesions, different cancer types or that have prognostic significance. Luo et al. (2001); Su et al. (2001); Henshall et al. (2003); and Wang et al. (2004). The first



gene expression profiling of malignant melanoma used a microarray containing probes for 8,150 cDNAs and identified genes that might be associated with aggressive tumor behavior. Bittner et al. (2000). Since the samples analyzed in their study did not include tissues containing normal or benign melanocytes, differentially expressed genes in malignant melanoma were not identified. In contrast to normal skin, melanocyte content in benign nevi is close to that in melanoma.

In another study, two pooled samples derived from either melanoma or benign nevi tissues were hybridized to a cDNA array and genes preferentially expressed in melanoma- or nevi-derived samples were found. Seykora et al. (2003). Other researchers used subtractive hybridization or analysis of SAGE libraries generated on melanoma cell lines, for monitoring gene expression in melanoma. Hipfel et al. (2000); and Weeraratna (2004). Recently, comparison of gene expression profiles of a few melanoma and melanocyte cell lines led to the identification of differentially expressed genes and pathways modulated in melanoma. Hoek et al. (2004). While these studies provide a solid foundation for melanoma genetics, there is no marker that can clearly differentiate melanoma from benign tissue. Several markers currently used such as tyrosinase and Mart-1 cannot discriminate between benign and malignant tissue. Takeuchi et al. (2004). Consequently, these markers have limited use in applications such as intra-operative, lymph-node-based staging of disease.

Difficulties in obtaining sufficient RNA samples from malignant melanoma and benign melanocyte lesions, tissue heterogeneity, and the presence of melanin in purified RNA remain the major challenges in these studies. In the study presented herein, total RNA isolated from 45 primary malignant melanomas, 18 benign skin nevi, and 7 normal skin tissues were hybridized on an Affymetrix Hu133A microarray containing 22,000 probe sets. A modified RNA extraction method was developed to produce melanin-free RNA samples that increased the microarray hybridization signals. Hierarchical clustering revealed distinct separation of the melanoma samples from benign and normal specimens. Significance Analysis of Microarray (SAM) method, *t-test* and percentile analysis identified 439 up-regulated (SEQ ID NOs: 29-467) and 511 down-regulated (SEQ ID NOs: 468-978) genes in the melanoma samples. Besides well-characterized genes such as me20m (gp100), melanocortin

receptor 1, and L1CAM, many novel genes previously unassociated with melanoma were identified including NTKR3 and PLAB. Pathway analysis of the differentially expressed genes revealed an over-representation of genes associated with neural tissue development and function, activation of amyloid processing and integrin signalling pathways. RT-PCR assays were performed to confirm the differential expression of the selected genes.

The methods provided have sufficient specificity and sensitivity to detect metastasis of melanoma. A comparison of the current methods available indicates that tradition methods of H&E and IHC are clinically acceptable whereas, prior to the current invention, PCR methods were unacceptable. Table 1 shows the drawbacks and advantages of current methods prior to the invention claimed herein.

Table 1

| Method | Sensitivity | Specificity |
|--------|-------------|-------------|
| H&E    | Low         | 100%        |
| IHC    | Low         | 100%        |
| PCR    | High        | Low         |

In the present invention, specificity is preferably at least 95%, more preferably, specificity is at least 97% and most preferably, specificity is at least 99% based on a comparison of H&E and IHC negative nodes. Preferably, sensitivity is at least at least 80%, more preferably sensitivity is at least 85% and most preferably, sensitivity is at least 90% based on a comparison of H&E and IHC positive nodes. Preferably, specificity and sensitivity are at least 97% based on a comparison of H&E and IHC negative nodes and at least 85% based on a comparison of H&E and IHC positive nodes, respectively.

Preferably, the pre-determined cut-off levels are at least two-fold over-expression in tissue having metastatic melanoma relative to benign melanocyte or normal tissue.

The preferred methods of the invention employ a rapid technique for extracting nucleic acids from a tissue sample and a method of amplifying and detecting nucleic acid fragments indicative of metastasis. The nucleic acid fragments qualitatively and quantitatively measure mRNA encoded by the Marker genes. Tissue samples include lymph node, both regional and sentinel, skin lesions and other biopsy material.

The methods provided herein allow for intra-operative detection of micrometastases allowing a physician to determine whether to excise additional lymph nodes and to immediately implement an appropriate treatment protocol. As shown in Table 2, if a LN is found to be positive for melanoma, regional LNs are excised and interferon therapy could be suggested. Standard biopsy methods can take over one week and a positive result requires additional surgery to remove LNs and there is a concomitant delay in interferon therapy.

Table 2

| Clinical Stage | 1° Tumor (T)     | LN       | Metastasis | Treatment   |
|----------------|------------------|----------|------------|---|
| Stage I        | T1: $\leq 1$ mm  | Negative | Absent     | Excision 1 cm   |
|                | T2: 1.01-2.00 mm | Negative | Absent     | Excision 1-2 cm   |
| Stage II       | T3: 2.01-4.00 mm | Negative | Absent     | Excision 2 cm   |
|                | T4: $> 4.01$ mm  | Negative | Absent     | Excision 2 cm   |
| Stage III      | Any thickness    | Positive | Absent     | Excision + complete LN dissection + interferon clinical trial |
| Stage IV       | Any thickness    | Positive | Present    | Interferon clinical trial, symptomatic therapy                |

It is important to adequately sample the tissue used to conduct the assay. This includes proper excision and processing of the tissue sample as well as extraction of RNA. Once obtained, it is important to process the tissue samples properly so that any cancerous cells present are detected.

In the most preferred embodiment of the invention, node sampling is also given attention both intra- and extra-operatively. Since the distribution of cancer cells in nodes is non-uniform, it is preferable that multiple sections of the node be sampled. Every identified SLN should be submitted for pathological evaluation. SLN material is ordinarily be fixed in formalin and examined as formalin fixed, paraffin embedded tissue sample. Equally representative parts of SLN are processed for molecular analysis (fresh tissue) and histology (fixed tissue). General LN sampling procedures are described in Cochran et al. (2001); and Cochran et al. (2004). One method for accomplishing both a molecular based test and an examination of the same node sample by pathology is to bisect the node through the longest diameter. Each half is then divided into at least four full-faced sections with at least one outer and inner

section for pathology as fixed material, and at least one outer and inner section for molecular testing. As the distribution of metastases and micrometastases is not uniform in nodes or other tissues, a sufficiently large sample should be obtained so that metastases will not be missed. One approach to this sampling issue in the present method is to homogenize a large tissue sample, and subsequently perform a dilution of the well-mixed homogenized sample to be used in subsequent molecular testing.

In the case of LN tissue samples, it is preferable to remove any adipose tissue prior to cellular disruption. Manual cell and tissue disruption can be by any means known in the art such as a disposable tissue grinder described in US Patent 4,715,545 or a commercial homogenizer such as Omni GLH1 15 with disposable probes (Omni International, Warrenton, VA). Homogenization time is within 1 to 2 minutes and is more preferably 30-45 sec. The sample can then be processed to purify the RNA prior to assaying and measuring Marker expression levels. Suitable RNA purification methods include columns such as (e.g., RNeasy mini column, QIAshredder, QIAGEN Inc., Valencia, CA, or a suitable substitute).

A variety of techniques are available for extracting nucleic acids from tissue samples. Typical commercially available nucleic acid extraction kits take at least 15 minutes to extract the nucleic acid. In the preferred intra-operative methods of the instant invention, nucleic acid is extracted in less than 8 minutes and preferably less than 6 minutes.

The successful isolation of intact RNA generally involves four steps: effective disruption of cells or tissue, denaturation of nucleoprotein complexes, inactivation of endogenous ribonuclease (RNase) and removal of contaminating DNA and protein. The disruptive and protective properties of guanidinium isothiocyanate (GITC) and  $\beta$ -mercaptoethanol ( $\beta$ -me) to inactivate the ribonucleases present in cell extracts make them preferred reagents for the first step. When used in conjunction with a surfactant such as sodium dodecylsulfate (SDS), disruption of nucleoprotein complexes is achieved allowing the RNA to be released into solution and isolated free of protein. Tissues are homogenized in the GITC-containing lysis buffer, addition of ethanol creates the appropriate conditions for RNA to bind to the silica membrane. Centrifugation can clear the lysate of precipitated proteins and cellular DNA and is

preferably performed through a column. RNA purification is preferably conducted on a spin column containing silica or other material.

RNA is precipitated via the spin column as described above and centrifugation times are preferably no greater than 30 sec. Typically, the sample is diluted with an equal volume of 70% ethanol and thoroughly mixed prior to applying to the column. After washing, the column is dried by centrifugation, and RNA is eluted in RNase free water and collected by centrifugation. The total time of this rapid protocol is less than 8 minutes and preferably less than 6 min.

In summary the rapid RNA extraction method involves the following steps:

- 10 obtaining a tissue sample;
- homogenizing the tissue to produce a homogenate;
- contacting the homogenate with a substrate containing, or to which is affixed, an RNA-binding material;
- allowing the RNA to bind to the RNA binding material;
- 15 washing the substrate under conditions sufficient to remove any contaminants, interferents and un-bound RNA; and
- eluting bound RNA from the substrate.

The reagents involved in this rapid extraction process can be those provided by the manufacturer or can be, for instance:

- 20 Lysis/Binding buffer (preferably, 4.5M GITC, 100mM NaPO<sub>4</sub>),
- Wash buffer I (preferably, 37% ethanol in 5M GITC, 20mM Tris-HCl),
- Wash buffer II (preferably, 80% ethanol in 20mM NaCl, 2mM Tris-HCl), and
- Nuclease-free sterile double distilled water for elution.

In one method, prior to the process for isolating nucleic acids described above, tissue samples are weighed and put into 8 or 14 ml polypropylene culture tubes and pre-cooled on dry ice. The frozen tissue samples are then divided into pieces of about 50 mg or less without being thawed. All buffers are those provided by QIAGEN in the RNeasy mini kit. A volume of homogenization (lysis) buffer is added to the tissue based on Table 3.

Table 3

| Tissue Weight (mg) | Homogenization buffer (ml) |
|--------------------|----------------------------|
| ≤ 100              | 2                          |
| 100-149            | 2                          |
| 150-199            | 3                          |
| 200-249            | 4                          |
| 250-299            | 5                          |
| 300-349            | 6                          |
| 350-399            | 7                          |
| 400-449            | 8                          |
| 450-499            | 9                          |
| 500-550            | 10                         |
| >550               | *                          |

\* Tissue above 550 mg is divided into equivalent parts and processed as individual samples. An alternative method to calculate lysis buffer volume for tissues over 100 mg is to add 1 ml per 50mg tissue; using 2 ml for tissues less than 100 mg.

5 The tissue sample is then homogenized for instance by the Omni GLH1 15 at a power setting to grade 6, Adaptor A1000 and disposable probes. The homogenate is then mixed with an equal volume of 70% ethanol and thoroughly mixed for instance by vortexing on a VWR Model G560 set at 10 speed (maximum) about 10 seconds or by pipetting 4-5 times. The homogenate/ethanol mixture is then applied to an RNeasy  
10 mini column mounted on a vacuum manifold in a volume in accordance with Table 4 so that a consistent amount of the original tissue (approximately 5 mg/column) is loaded thus producing comparable RNA yields for each tissue sample.

Table 4

| Tissue weight (mg) | Volume homogenate/ethanol mix (μl) (recommended) |
|--------------------|--|
| 30-39              | 700  |
| 40-49              | 500  |
| 50-59              | 400  |
| 60-69              | 350  |
| 70-79              | 300  |
| 80-89              | 250  |
| 90-99              | 225  |
| > 100              | 200  |

A vacuum is then applied to the column to remove the liquid. The vacuum is  
15 stopped and two washes of 700 ml are applied, first with RWI buffer and second with

RPE buffer each removed by filtration. Vacuum is at 800-1200 mBar in each case. The column is then placed into a 1.5 ml collection tube and centrifuged in an Eppendorf 5415D centrifuge at 13,200 rpm for 30 seconds to dry. The column is transferred to a new 1.5 ml collection tube. Fifty  $\mu$ l RNase-free water is directly  
5 added to the membrane and the column is centrifuged in an Eppendorf 541 5D centrifuge for 30 seconds at 13,200 rpm to elute the RNA. The RNA quality is determined with an Agilent Bioanalyzer and the RNA is stored at -70  $^{\circ}$ C.

Melanin can negatively impact the efficiency of reversed transcription and amplification reactions. Accordingly, a melanin removal process is undertaken when  
10 the sample is suspected of containing a significant amount of melanin (as in the case of samples of a primary melanoma or benign skin nevi) and is less of a concern when performing the assay on a SLN since melanocyte content is low. If necessary, melanin is removed to enhance reverse transcription and/or nucleic acid amplification.

Typically, melanin is removed during the filtration steps provided above. In the  
15 case of tissue with high melanin concentration, less tissue should be used, approximately 5 mg per Qiagen RNeasy mini column.

If another method is used that results in residual melanin in the sample, removal involves the use of a matrix employing a polymer bead system such as Bio-Gel P-60 (Bio-Rad Laboratories, Hercules, CA). Such a method is described by Satyamoorthy  
20 et al. (2002). Essentially, this method involves preparing a 50% (w/v) mixture of the Bio-Gel material in 10 mM sodium acetate (pH 4.2). About 300  $\mu$ l of the mixture are placed in a micro-centrifuge tube and centrifuged at 1000rpm for 1 min. The supernatant is discarded and the beads are placed in a mini-column or similar vessel. Homogenate is then passed through the vessel containing the beads (after first  
25 incubating them in the vessel). The supernatant is collected. Further washing of the beads with additional 100  $\mu$ l aliquots of 10 mM sodium acetate can be used to capture additional volumes of melanin-free sample if necessary for adequate assay volume. The dark melanin will be clearly visible on the beads retained in the vessel. Other silica-based filters can also be used to remove the melanin pigment as described by  
30 Wang et al. (2001).

An important aspect of the intra-operative methods of the invention is rapid Marker detection. Provided that such methods can be conducted within a period acceptable for an intra-operative assay (i.e., no more than about 35 minutes), any reliable, sensitive, and specific method can be used.

5 In the case of measuring mRNA levels to determine gene expression, assays can be by any means known in the art and include methods such as PCR, Rolling Circle Amplification (RCA), Ligase Chain Reaction (LCR), Strand Displacement Amplification (SDA), Nucleic Acid Sequence Based Amplification (NASBA), and others. The rapid molecular diagnostics involved are most preferably quantitative  
10 PCR methods, including QRT-PCR. Detection can be by any method known in the art including microarrays, gene chips and fluorescence.

A typical PCR includes multiple amplification steps, or cycles that selectively amplify target nucleic acid species. A typical PCR includes three steps: a denaturing step in which a target nucleic acid is denatured; an annealing step in which a set of  
15 PCR primers (forward and backward primers) anneal to complementary DNA strands; and an elongation step in which a thermostable DNA polymerase elongates the primers. By repeating this step multiple times, a DNA fragment is amplified to produce an amplicon, corresponding to the target DNA sequence. Typical PCR includes 20 or more cycles of denaturation, annealing and elongation. Often, the  
20 annealing and elongation steps can be performed concurrently, in which case the cycle contains only two steps.

In the preferred inventive method, employing RT-PCR, the RT-PCR amplification reaction is conducted in a time suitable for intra-operative diagnosis, the lengths of each of these steps can be in the seconds range, rather than minutes. Specifically,  
25 with certain new thermal cyclers being capable of generating a thermal ramp rate of at least about 5C° per second, RT-PCR amplifications in 30 minutes or less are used. More preferably, amplifications are conducted in less than 25 minutes. With this in mind, the following times provided for each step of the PCR cycle do not include ramp times. The denaturation step may be conducted for times of 10 seconds or less.  
30 In fact, some thermal cyclers have settings for "0 seconds" which may be the optimal duration of the denaturation step. That is, it is enough that the thermal cycler reaches



the denaturation temperature. The annealing and elongation steps are most preferably less than 10 seconds each, and when conducted at the same temperature, the combination annealing/elongation step may be less than 10 seconds. Some homogeneous probe detection methods, may require a separate step for elongation to maximize rapid assay performance. In order to minimize both the total amplification time and the formation of non-specific side reactions, annealing temperatures are typically above 50°C. More preferably annealing temperatures are above 55°C.

A single combined reaction for RT-PCR, with no experimenter intervention, is desirable for several reasons: (1) decreased risk of experimenter error; (2) decreased risk of target or product contamination; and (3) increased assay speed. The reaction can consist of either one or two polymerases. In the case of two polymerases, one of these enzymes is typically an RNA-based DNA polymerase (reverse transcriptase) and one is a thermostable DNA-based DNA polymerase. To maximize assay performance, it is preferable to employ a form of "hot start" technology for both of these enzymatic functions. US Patents 5,411,876 and 5,985,619 provide examples of different "hot start" approaches. Preferred methods include the use of one or more thermoactivation methods that sequester one or more of the components required for efficient DNA polymerization. US Patents 5,550,044 and 5,413,924 describe methods for preparing reagents for use in such methods. US Patent 6,403,341 describes a sequestering approach that involves chemical alteration of one of the PCR reagent components. In the most preferred embodiment, both RNA- and DNA-dependent polymerase activities reside in a single enzyme. Other components that are required for efficient amplification include nucleoside triphosphates, divalent salts and buffer components. In some instances, non-specific nucleic acid and enzyme stabilizers may be beneficial.

In the preferred RT-PCR, the amounts of certain reverse transcriptase and the PCR components are atypical in order to take advantage of the faster ramp times of some thermal cyclers. Specifically, the primer concentrations are very high.

Typical gene-specific primer concentrations for reverse transcriptase reactions are less than about 20 nM. To achieve a rapid reverse transcriptase reaction on the order of one to two minutes, the reverse transcriptase primer concentration is raised to

greater than 20 nM, preferably at least about 50 nM, and typically about 100 nM. Standard PCR primer concentrations range from 100 nM to 300 nM. Higher concentrations may be used in standard PCR to compensate for  $T_m$  variations. However, for the purposes herein, the referenced primer concentrations are for  
5 circumstances where no  $T_m$  compensation is needed. Proportionately higher concentrations of primers may be empirically determined and used if  $T_m$  compensation is necessary or desired. To achieve rapid PCR, the PCR primer concentrations typically are greater than 250 nM, preferably greater than about 300 nM and typically about 500 nM.

10 Commercially used diagnostics also preferably employ one or more internal positive control that confirms the operation of a particular amplification reaction in case of a negative result. Potential causes of false negative results that must be controlled in an RT-PCR include: inadequate RNA quantity, degradation of RNA, inhibition of RT and/or PCR and experimenter error.

15 In the case of measuring protein levels to determine gene expression, any method known in the art is suitable provided it results in adequate specificity and sensitivity. For example, protein levels can be measured by binding to an antibody or antibody fragment specific for the protein and measuring the amount of antibody-bound protein. Antibodies can be labeled by radioactive, fluorescent or other detectable  
20 reagents to facilitate detection. Methods of detection include, without limitation, enzyme-linked immunosorbent assay (ELISA) and immunoblot techniques.

The invention provides specificity and sensitivity sufficient to identify a malignant melanocyte in a tissue sample. The methods determine expression of particularly Marker genes by measuring mRNA encoded by the Markers. The preferred Markers  
25 of the invention display at least a two-fold over-expression in tissue having malignant melanocytes relative to benign melanocyte or normal tissue. The results presented herein show that a primary Marker is insufficient to provide clinically relevant information but, when combined with one or more secondary Markers, the information obtained compares to the "gold standard" of H&E and IHC upon which  
30 clinicians currently rely. Tertiary Markers and control genes can augment the primary and secondary Markers to further increase specificity and/or sensitivity.

As described in the following Examples, the Markers were identified by the protocol depicted in Figure 1. Thus, the invention provides a method for identifying melanoma-specific Markers by following the protocol in Figure 1 and the Examples provided herein.

5       The primary Marker can be PLAB and is defined herein as the gene encoding any variant, allele etc. including SEQ ID NO: 1. PLAB is also described by Paralkar et al. (1998) and represented by Accession No. AF003934. PLAB is linked to the pathogenesis of prostate cancer (Liu et al (2003); Karan et al. (2003); and Nakamura et al. (2003); US Patent Nos. 5,994,102; 6,107,476; 6,465,181; 6,500,638; 6,521,227; 10   US Patent Publication Nos. 2002/0048784; 2003/0013097; and 2003/0059431) and colorectal cancer (Brown et al. (2003); Buckhaults et al. (2001); and US Patent Publication No. 2002/0160382).

      The secondary Marker is LICAM and is defined herein as the gene encoding any variant, allele etc. including SEQ ID NO: 2. LICAM is also described by Haspel et al 15   (2003); and US Patent Nos. 5,872,225; and 5,969,124 and is represented by Accession No. NM\_000425.

      The invention further provides tertiary markers that fall into several functional categories. Thus, additional Markers can be used that are found in these functional categories. As described in more detail in the Examples, melanoma-specific 20   up-regulated genes fall into the functional categories of neural tissue development and cell cycle control and melanoma-specific down-regulated genes fall into the functional categories of tissue development and cell differentiation.

      The tertiary Markers include SEQ ID NOs: 3, 29-978 and 999. A number of tertiary markers are described in Table 5 and all are summarized in Table 15.

25       NTRK3 is described by Strausberg et al. (2002); Marchetti et al. (2003); Hisaoka et al. (2002); McGregor et al. (1999); Ryden et al. (1996); US Patent Nos. 5,348,856; 5,844,092; 5,910,574; and US Patent Publication Nos. 2002/0155480; and 2003/014283 and is represented by Accession No. BC013693 or S76476.1. NTRK3 is also defined as the gene encoding mRNA recognized by the primer/probe sets SEQ 30   ID NOs: 16-18.

Tyrosinase is described by Mandelcorn-Monson et al. (2003); and US Patent No. 6,153,388 and is represented by Accession No. NM\_000372. Tyrosinase is also defined as the gene encoding mRNA recognized by the primer/probe sets SEQ ID NOs: 19-21.

5

Table 5

| Gene                              | Reference   | Accession # |
|-----------------------------------|---|-------------|
| PBGD                              | Raich et al. (1986)   | NM_000190   |
| CITED1                            | Fenner et al. (1998)  | NM_004143   |
| PEX6                              | Raas-Rothschild et al. (2002)                                     | NM_000287   |
| CAPG                              | Van Impe et al. (2003)  | NM_001747   |
| DUSP4                             | Smith et al. (1997)   | NM_001394   |
| GDF1                              | Ducy et al. (2000)  | NM_001492   |
| E2-EpF                            | Liu et al. (1992)   | NM_014501   |
| me20m                             | Maresh et al. (1994)  | U01874      |
| CDH3                              | Patel et al. (2003)   | NM_001793   |
| SMARCD3                           | Ring et al. (1998)  | NM_003078   |
| PKM2                              | Luftner et al. (2003)   | NM_002654   |
| GPI                               | Tsutsumi et al. (2003)  | NM_000175   |
| Pig10                             | Polyak et al. (1997)  | AF010413    |
| CPEB1                             | Welk et al. (2001)  | NM_030594   |
| HOXHB9                            | Catala et al. (2002)  | AI738662    |
| Truncated calcium binding protein | The Washington University-Merck EST Project Hillier et al. (1995) | N30649      |
| SAAS                              | Kikuchi et al. (2003)   | NM_013271   |
| HS1-2                             | Edgar et al. (2002)   | NM_007011   |
| HCN2                              | Stieber et al. (2003)   | NM_001194   |
| MBP                               | Kamholz et al. (1996)   | M13577      |
| AD3LPAD5                          | Li et al. (1995)  | U34349      |
| PLOD3                             | Wang et al. (2002)  | NM_001084   |
| MC1R                              | Salazar-Onfray et al. (2002)                                      |             |
| MIF                               | Shimizu et al. (1999)   | NM_002415   |
| HOXB7                             | Care et al. (1996)  | NM_004502   |
| AIM1                              | Ray et al. (1997)   | XM_166300   |
| EpHB6                             | Hafner et al. (2003)  | NM_004445   |
| AKT1                              | Majumder et al. (2004)  | NM_005163   |
| AKT2                              | Gosmanov et al. (2004)  | NM_001626   |
| AKT3                              | Xu et al. (2003)  | NM_005465   |
| APH-1A                            | Xu et al. (2003)  | NM_016022   |
| APP                               | Masters et al. (1985)   | NM_201414   |
| BACE                              | Pastorino et al. (2004)   | NM_138973   |
| BACE2                             | Pastorino et al. (2004)   | NM_012104   |
| CAPN1                             | Altnauer et al. (2004)  | NM_005186   |
| CAPN2                             | Alexa et al. (2004)   | NM_001748   |

|         |                          |           |
|---------|--------------------------|-----------|
| CDK5    | Qi et al. (2004)         | NM 004935 |
| CDK5R1  | Kam et al. (2004)        | NM 003885 |
| CSNK1A1 | Burzio et al. (2002)     | NM 001892 |
| CSNK1D  | Li et al. (2004)         | NM 139062 |
| CSNK1E  | Swiatek et al. (2004)    | NM 152221 |
| CSNK2A1 | Hilgard et al. (2004)    | NM 001895 |
| CSNK2A2 | Szebeni et al. (2003)    | NM 001896 |
| CSN2K2B | Lee et al. (2004)        | NM 001320 |
| GSK3B   | Chen et al. (2003)       | NM 182946 |
| MAPK1   | Nishihara et al. (2004)  | NM 138957 |
| MAPK14  | Bendotti et al. (2004)   | NM 139014 |
| MAPK3   | Nishihara et al. (2004)  | NM 002746 |
| MAPT    | Yu et al. (2004)         | NM 016841 |
| NCSTN   | Shirotani et al. (2004)  | NM 015331 |
| PEN2    | Marlow et al. (2003)     | NM 172341 |
| PRKACA  | Sakwe et al. (2004)      | NM 207518 |
| PRKACB  | Dwivedi et al. (2004)    | NM 002731 |
| PRKACG  | Zhang et al. (2004)      | NM 002732 |
| PRKARIA | Gronholm et al. (2003)   | NM 212472 |
| PRKAR2A | MacDougall et al. (2003) | NM 004157 |
| PRKAR2B | Dwivedi et al. (2004)    | NM 002736 |
| PRKCE   | Schechtman et al. (2004) | NM 005400 |
| PSEN1   | Pitsi et al. (2004)      | NM 000021 |
| PSEN2   | Zatti et al. (2004)      | NM 012486 |
| PSFL    | Clark et al. (2003)      | NM 031301 |
| ABL1    | Gustafson et al. (2004)  | NM 007313 |
| ACK1    | Ahmed et al. (2004)      | NM 005781 |
| ACTN4   | Menez et al. (2004)      | NM 004924 |
| ARF1    | Kadaja et al. (2004)     | NM 001658 |
| ARPC1B  | Kaneda et al. (2002)     |           |
| BCAR3   | Clark et al. (2003)      | NM 003567 |
| BRAF    | Sasaki et al. (2004)     | NM 004333 |
| CDC42   | Chen et al. (2004)       | NM 044472 |
| CRK     | Stoletov et al. (2004)   | NM 016823 |
| CRKL    | Zhang et al. (2003)      | NM 005207 |
| DDEF1   | Oda et al. (2003)        | NM 018482 |
| DOCK1   | Grimsley et al. (2004)   | NM 001380 |
| FYN     | Lee et al. (2004)        | NM 153048 |
| GIT1    | Haendeler et al. (2003)  | NM 014030 |
| GRB2    | Zhou et al. (2004)       | NM 203506 |
| GRF2    | Arozarena et al. (2004)  | NM 006909 |
| HRAS    | Nomura et al. (2004)     | NM 005343 |
| JUN     | Schmuth et al. (2004)    | NM 002228 |
| KRAS2   | Qi et al. (2004)         | NM 033360 |
| MAP2K1  | Rhee et al. (2004)       | NM 002755 |

|         |                            |           |
|---------|----------------------------|-----------|
| MAP2K2  | Chen et al. (2004)         | NM 030662 |
| MAP2K4  | Woo et al. (2004)          | NM 003010 |
| MAP3K11 | Zhang et al. (2004a)       | NM 002419 |
| MAPK8   | Fujii et al. (2004)        | NM 139049 |
| MYLK    | Oury et al. (2004)         | NM 053032 |
| NRAS    | Reifenberger et al. (2004) | NM 002524 |
| PAK1    | Sells et al. (1997)        | HSU24152  |
| PAK2    | Kirchhoff et al. (2004)    | NM 002577 |
| PAK3    | Kitano et al. (2003)       | NM 002578 |
| PAK4    | Barac et al. (2004)        | NM 005884 |
| PAK6    | Ching et al. (2003)        | NM 020168 |
| PAK7    | Jaffer et al. (2002)       | NM 020341 |
| PTK2    | Golubovskaya et al. (2004) | NM 005607 |
| PXN     | Saito et al. (2004)        | NM 002859 |
| RAC1    | Pontow et al. (2004)       | NM 198829 |
| RAF1    | Akula et al. (2004)        | NM 002880 |
| RAP1A   | Nomura et al. (2004)       | NM 002884 |
| RAP2B   | Evellin et al. (2002)      | NM 002886 |
| SHC1    | Yannoni et al. (2004)      | NM 183001 |
| SOS1    | Buchs et al. (2004)        | NM 005633 |
| SRC     | Encinas et al. (2004)      | NM 198291 |
| TLN1    | Tremuth et al. (2004)      | NM 006289 |
| VASP    | Tokuo et al. (2004)        | NM 003370 |
| VCL     | Izard et al. (2004)        | NM 003373 |
| WASPIP  | Luthi et al. (2003)        | NM 003387 |
| ZYX     | Li et al. (2004)           | NM 003461 |

Tertiary Markers are able to replace and/or supplement primary or secondary Markers provided that the resulting assays have adequate sensitivity and specificity.

The specificity of any given amplification-based molecular diagnostic relies heavily, but not exclusively, on the identity of the primer sets. The primer sets are pairs of forward and reverse oligonucleotide primers that anneal to a target DNA sequence to permit amplification of the target sequence, thereby producing a target sequence-specific amplicon. The primers must be capable of amplifying Markers of the disease state of interest. In the case of the instant invention, these Markers are directed to melanoma.

The reaction must also contain some means of detection of a specific signal. This is preferably accomplished through the use of a reagent that detects a region of DNA sequence derived from polymerization of the target sequence of interest. Preferred reagents for detection give a measurable signal differential when bound to a specific

nucleic acid sequence of interest. Often, these methods involve nucleic acid probes that give increased fluorescence when bound to the sequence of interest. Typically, the progress of the reactions of the inventive methods are monitored by analyzing the relative rates of amplicon production for each PCR primer set.

5       The invention further includes primer/probe sets and their use in the claimed methods. The sequences are:

SEQ ID NO:4 (PLAB forward primer) ggcagaatcttcgtccgca  
 SEQ ID NO:5 (PLAB reverse primer) ggacagtgggtcccggtg  
 SEQ ID NO:6 (PLAB probe) cccagctggagttgcacttgcggcc  
 10   SEQ ID NO:7 (PLAB upper primer) gaacaccgacctcgtccc  
 SEQ ID NO:8 (PLAB lower primer) ggcggcccgagagata  
 SEQ ID NO:9 (PLAB probe) cgccagaagtgcggctgggattt  
 SEQ ID NO:10 (LICAM forward) gctgggactgggaacagaact  
 SEQ ID NO:11 (LICAM Reverse) ggagcagagatggcaaagaaa  
 15   SEQ ID NO:12 (LICAM probe) ttccccaccatctgtctgt  
 SEQ ID NO:13 (LICAM upper) ccacagatgacatcagcctcaa  
 SEQ ID NO:14 (LICAM lower) ggtcacaccagctcttctt  
 SEQ ID NO:15 (LICAM probe) tggcaagcccgaagtgcagttcctt  
 SEQ ID NO:16 (NTRK3 primer) gccccggcacccttta  
 20   SEQ ID NO:17 (NTRK3 primer) aacctgccagtggtggat  
 SEQ ID NO:18 (NTRK3 probe) cagatgggtgttttc  
 SEQ ID NO:19 (Tyr upper) actcagcccagcatcattcttc  
 SEQ ID NO:20 (Tyr lower) atggctgtgtactctccaatc  
 SEQ ID NO:21 (Tyr probe) ctctcctcttggcagattgtctgtagctt  
 25   SEQ ID NO:22 (PBGD upper) ccacacacagcctactttccaa  
 SEQ ID NO:23 (PBGD lower) taccacgcgaatcacttca  
 SEQ ID NO:24 (PBGD probe) aacggcaatgcggctgcaacggcggaatt

Monitoring amplicon production may be achieved by a number of detection reagents and methods, including without limitation, fluorescent primers, and  
 30   fluorogenic probes and fluorescent dyes that bind double-stranded DNA. Molecular beacons, Scorpions, and other detection schemes may also be used. A common

method of monitoring a PCR employs a fluorescent hydrolysis probe assay. This method exploits the 5' nuclease activity of certain thermostable DNA polymerases (such as Taq or Tfi DNA polymerases) to cleave an oligomeric probe during the PCR process.

- 5       The invention further provides amplicons obtained by PCR methods utilized in the inventive methods. These amplicons include the following:

SEQ ID NO:25 (PLAB Amplicon)

gaacaccgacctcgtcccggcccctgcagtcggtatactcacgccagaagtgcggctgggatccggcgccacctgca  
cctgcgtatctctcgggcccgc

- 10      SEQ ID NO:26 (LI CAM Amplicon)

ccacagatgacatcagcctcaagtgtgaggccagtggaagcccgaagtgcagttccgctggacgagggatggtgtcca  
cttcaaaccaaggaagagctgggtgtgacc

SEQ ID NO:27 (tyrosinase Amplicon)

actcagcccagcatcattcttctcttggcagattgtctgtagccgattggaggagtacaacagccat

- 15      SEQ ID NO:28 (PBGD Amplicon)

ccacacacagcctactttccaagcggagccatgtctgttaacggcaatgcggctgcaacggcggaagaaaacagcccaa  
agatgagagtgattcgcgtgggta

- 20       The oligomer is selected to anneal to the amplified target sequence under elongation conditions. The probe typically has a fluorescent reporter on its 5' end and a fluorescent quencher of the reporter at the 3' end. So long as the oligomer is intact, the fluorescent signal from the reporter is quenched. However, when the oligomer is digested during the elongation process, the fluorescent reporter is no longer in proximity to the quencher. The relative accumulation of free fluorescent reporter for a given amplicon may be compared to the accumulation of the same amplicons for a control sample and/or to that of a control gene, such as, without limitation,  $\beta$ -Actin or PBGD to determine the relative abundance of a given cDNA product of a given RNA in a RNA population. Products and reagents for the fluorescent hydrolysis probe assay are readily available commercially, for instance from Applied Biosystems.

- 25       Suitable detection reagents are commonly referred to as "Scorpions" and are described in US Patents 6,326,145 and 5,525,494. These reagents include one or more molecules comprising a tailed primer and an integrated signaling system. The
- 30



primer has a template binding region and a tail comprising a linker and a target binding region. The target binding region in the tail hybridizes to complementary sequence in an extension product of the primer. This target specific hybridization event is coupled to a signaling system wherein hybridization leads to a detectable change. In PCR the target binding region and the tail region are advantageously arranged such that the tail region remains single stranded, i.e. uncopied. Thus the tail region is non-amplifiable in the PCR amplification products. The linker comprises a blocking moiety that prevents polymerase mediated chain extension on the primer template.

10 The most preferred detection reagents are TaqMan® probes (Roche Diagnostics, Branchburg, NJ) and they are described in US Patents 5,210,015; 5,487,972; and 5,804,375. Essentially, these probes involve nucleic acid detection by virtue of the separation of a fluor-quencher combination on a probe through the 5'-3' exonuclease activity of the polymerase used in the PCR. Any suitable fluorophore can be used for  
15 any of the Markers or controls. Such fluorophores include, without limitation, Texas Red, Cal Red, Fam, Cy3 and Cy5. In one embodiment, the following fluorophores correspond to the noted Markers: PLAB: Fam; LICAM: Texas Red or Cal Red, tyrosinase: Cl; PBGD: Cy5.

Equipment and software also are readily available for controlling and monitoring  
20 amplicon accumulation in PCR and QRT-PCR including the Smart Cycler thermocycler commercially available from Cepheid of Sunnyvale, California, and the ABI Prism 7700 Sequence Detection System, commercially available from Applied Biosystems.

In the case of gene expression assays, it is preferable to use a gene constitutively  
25 expressed in the tissue of interest. PBGD is commonly used as an internal control due to several factors: it contains no known pseudogenes in humans, it is constitutively expressed in human tissues and it is expressed at a relatively low level and therefore is less likely to cause inhibition of the amplification of target sequences of interest. Use of PBGD as a control minimizes or eliminates reporting erroneous results arising from  
30 all potential sources of false negative results.

In the commercialization of the described methods for QRT-PCR certain kits for detection of specific nucleic acids are particularly useful. In one embodiment, the kit includes reagents for amplifying and detecting Markers. Optionally, the kit includes sample preparation reagents and or articles (e.g., tubes) to extract nucleic acids from lymph node tissue. The kits may also include articles to minimize the risk of sample contamination (e.g., disposable scalpel and surface for lymph node dissection and preparation).

In a preferred kit, reagents necessary for the one-tube QRT-PCR process described above are included such as reverse transcriptase, a reverse transcriptase primer, a corresponding PCR primer set (preferably for Markers and controls), a thermostable DNA polymerase, such as Taq polymerase, and a suitable detection reagent(s), such as, without limitation, a scorpion probe, a probe for a fluorescent hydrolysis probe assay, a molecular beacon probe, a single dye primer or a fluorescent dye specific to double-stranded DNA, such as ethidium bromide. The primers are preferably in quantities that yield the high concentrations described above. Thermostable DNA polymerases are commonly and commercially available from a variety of manufacturers. Additional materials in the kit may include: suitable reaction tubes or vials, a barrier composition, typically a wax bead, optionally including magnesium; reaction mixtures (typically 10X) for the reverse transcriptase and the PCR stages, including necessary buffers and reagents such as dNTPs; nuclease-or RNase-free water; RNase inhibitor; control nucleic acid(s) and/or any additional buffers, compounds, co-factors, ionic constituents, proteins and enzymes, polymers, and the like that may be used in reverse transcriptase and/or PCR stages of QRT-PCR. Optionally, the kits include nucleic acid extraction reagents and materials. Instructions are also preferably included in the kits.

The following examples are provided to illustrate but not limit the claimed invention. AU references cited herein are hereby incorporated herein by reference.

### **Example 1**

#### **Tissue Preparation**

Fresh frozen malignant melanoma, benign skin nevi, normal skin, melanoma lymph node metastasis and melanoma-free lymph node samples were obtained from  
5 Genomics Collaborative, Inc. (Cambridge, MA), Asterand (Detroit, MI), Clinomics (Pittsfield, MA) and Proteogenex (Los Angeles, CA), Ardais (Lexington, MA) and Impath (Westborough, MA). All tissue vendors declared that tissue specimens used in the study were collected according to an Institutional Review Board approved protocol of corresponding hospitals and principles of bioethics. Patients' demographic  
10 and pathology information was also collected. The histopathological features of each sample were reviewed to confirm diagnosis, and to estimate sample preservation and tumor content.

Melanoma and benign nevi primary tissues chosen for microarray analysis had melanocyte content greater than 50% with no mixed histology. Melanoma positive  
15 lymph nodes were collected from malignant melanoma patients; diagnosis of melanoma was confirmed by H&E in combination with IHC (S100 and HMB45). Melanoma free lymph nodes derived from patients that did not have melanoma in their clinical history and absence of melanoma was confirmed by H&E and IHC using antibodies for S100 and HMB45.

20 RNA from a total of 70 primary tissue samples was used for gene expression profiling and melanoma specific gene identification. Samples included 45 primary malignant melanoma, 18 benign skin nevi, and 7 normal skin tissues. The majority of primary melanomas included in the study represent early stage of disease and have thickness less than 4 mm, which is consistent with the standard melanoma patient  
25 population. Aitken et al. (2004). Patient demographic, clinical and pathology characteristics are presented in Table 6 and summarized in Table 7.

In addition, 77 malignant melanoma LN metastasis and 18 melanoma-free LN tissue samples were used for one-step quantitative PCR assay. Melanoma positive lymph nodes included axillary, cervical and inguinal lymph nodes with metastasis  
30 derived from epithelioid and spindle cell primary melanomas. Out of 18 melanoma free LN, 10 were collected from other cancer patients but no cancer cells were found in these nodes by pathologists and 8 LN were from non-malignant lesions.

Table 6

| Sample ID | Age | Gender | Race  | Diagnosis                | Location              | T Stage | N&M Stage | Clark level |
|-----------|-----|--------|-------|--------------------------|-----------------------|---------|-----------|-------------|
| 430MM     | n/a | F      | Cau   | normal skin              | trunk                 |         |           |             |
| 431MM     | n/a | F      | Cau   | normal skin              | trunk                 |         |           |             |
| 432MM     | n/a | F      | Cau   | normal skin              | trunk                 |         |           |             |
| 433MM     | n/a | F      | Cau   | normal skin              | trunk                 |         |           |             |
| 435MM     | n/a | F      | Cau   | normal skin              | trunk                 |         |           |             |
| 437MM     | n/a | F      | Cau   | normal skin              | trunk                 |         |           |             |
| 485MM     | 37  | M      | Cau   | normal skin              | skin, NOS             |         |           |             |
| 487MM     | 35  | F      | Cau   | atypical nevus, mild     | face                  |         |           |             |
| 489MM     | 56  | F      | Cau   | compound nevus           | face                  |         |           |             |
| 490MM     | 16  | F      | Cau   | compound nevus           | scalp & neck          |         |           |             |
| 491MM     | 15  | M      | Cau   | compound nevus           | trunk                 |         |           |             |
| 493MM     | 35  | F      | Cau   | compound nevus           | trunk                 |         |           |             |
| 495MM     | 18  | F      | Cau   | benign nevus, NOS        | trunk                 |         |           |             |
| 496MM     | 21  | F      | Cau   | intradermal nevus        | lower limb & hip      |         |           |             |
| 497MM     | 12  | M      | Cau   | intradermal nevus        | lower limb & hip      |         |           |             |
| 498MM     | 44  | F      | Cau   | benign nevus, NOS        | trunk                 |         |           |             |
| 499MM     | 48  | F      | Cau   | benign nevus, NOS        | face                  |         |           |             |
| 500MM     | 65  | M      | Cau   | intradermal nevus        | trunk                 |         |           |             |
| 501MM     | 30  | M      | Cau   | compound nevus           | lower limb & hip      |         |           |             |
| 502MM     | 20  | F      | Cau   | compound nevus           | trunk                 |         |           |             |
| 503MM     | 35  | M      | Cau   | intradermal nevus        | lower limb & hip      |         |           |             |
| 504MM     | 23  | M      | Cau   | compound nevus           | trunk                 |         |           |             |
| 507MM     | 53  | M      | Cau   | atypical nevus, moderate | trunk                 |         |           |             |
| 508MM     | 28  | M      | Cau   | compound nevus           | trunk                 |         |           |             |
| 509MM     | 43  | M      | Cau   | intradermal nevus        | trunk                 |         |           |             |
| 392MM     | 58  | F      | Cau   | epithelioid melanoma     | trunk                 | T3      | N0M0      | 4           |
| 397MM     | 51  | F      | Cau   | epithelioid melanoma     | lower limb & hip      | T2      | N0M0      | 3           |
| 405MM     | 46  | M      | Cau   | epithelioid melanoma     | upper limb & shoulder | T2      | N0M0      | 3           |
| 407MM     | 64  | F      | Cau   | epithelioid melanoma     | trunk                 | T1      | N0M0      | 2           |
| 409MM     | 54  | F      | Cau   | epithelioid melanoma     | scalp & neck          | T2      | N0M0      | 3           |
| 440MM     | 61  | M      | Cau   | malignant melanoma, NOS  | lower limb & hip      | T1      | N0M0      | 2           |
| 441MM     | 78  | M      | Cau   | spindle cell melanoma    | face                  | T4      | N0M0      | 5           |
| 442MM     | 52  | M      | Cau   | malignant melanoma, NOS  | upper limb & shoulder | T2      | N0M0      | 3           |
| 443MM     | 51  | F      | Cau   | spindle cell melanoma    | trunk                 | T2      | N0M0      | 3           |
| 444MM     | 49  | F      | Cau   | spindle cell melanoma    | lower limb & hip      | T3      | N0M0      | 4           |
| 445MM     | 76  | F      | Cau   | malignant melanoma, NOS  | upper limb & shoulder | T3      | N0M0      | 4           |
| 446MM     | 86  | M      | Cau   | malignant melanoma, NOS  | scalp & neck          | T1      | N0M0      | 2           |
| 447MM     | 48  | M      | Cau   | epithelioid melanoma     | skin, NOS             | T3      | N0M0      | 4           |
| 448MM     | 72  | F      | Cau   | epithelioid melanoma     | upper limb & shoulder | T2      | N0M0      | 3           |
| 449MM     | 62  | M      | Asian | epithelioid melanoma     | lower limb & hip      | T3      | N1M0      | n/a         |
| 450MM     | 90  | F      | Cau   | epithelioid melanoma     | upper limb & shoulder | T4      | N1M1      | n/a         |
| 452MM     | 43  | M      | Cau   | epithelioid melanoma     | skin, NOS             | T3      | N0M0      | n/a         |
| 453MM     | 48  | F      | Cau   | epithelioid melanoma     | trunk                 | T3      | N0M0      | n/a         |
| 454MM     | 69  | M      | Cau   | epithelioid melanoma     | upper limb & shoulder | T3      | N0M0      | n/a         |
| 455MM     | 55  | M      | Cau   | malignant melanoma, NOS  | skin, NOS             | T2      | N0M0      | n/a         |
| 456MM     | 63  | M      | Cau   | malignant melanoma, NOS  | lower limb & hip      | T2      | N0M0      | 3           |

|       |    |   |     |                         |                       |    |      |   |
|-------|----|---|-----|-------------------------|-----------------------|----|------|---|
| 457MM | 69 | M | Cau | spindle cell melanoma   | trunk                 | T1 | N0M0 | 2 |
| 459MM | 86 | F | Cau | malignant melanoma, NOS | lower limb & hip      | T2 | N0M0 | 3 |
| 460MM | 64 | M | Cau | malignant melanoma, NOS | upper limb & shoulder | T3 | N0M0 | 4 |
| 461MM | 66 | M | Cau | epithelioid melanoma    | trunk                 | T1 | N0M0 | 2 |
| 463MM | 58 | M | Cau | malignant melanoma, NOS | trunk                 | T1 | N0M0 | 2 |
| 464MM | 53 | M | Cau | epithelioid melanoma    | face                  | T2 | N0M0 | 3 |
| 465MM | 77 | F | Cau | epithelioid melanoma    | upper limb & shoulder | T3 | N0M0 | 4 |
| 466MM | 79 | F | Cau | malignant melanoma, NOS | upper limb & shoulder | T1 | N0M0 | 2 |
| 468MM | 86 | F | Cau | spindle cell melanoma   | upper limb & shoulder | T2 | N0M0 | 3 |
| 469MM | 43 | F | Cau | malignant melanoma, NOS | scalp & neck          | T1 | N0M0 | 2 |
| 470MM | 81 | M | Cau | malignant melanoma, NOS | upper limb & shoulder | T2 | N0M0 | 3 |
| 472MM | 38 | F | Cau | spindle cell melanoma   | upper limb & shoulder | T1 | N0M0 | 2 |
| 473MM | 69 | F | Cau | malignant melanoma, NOS | upper limb & shoulder | T1 | N0M0 | 3 |
| 475MM | 77 | F | Cau | malignant melanoma, NOS | face                  | T3 | N0M0 | 4 |
| 476MM | 87 | F | Cau | spindle cell melanoma   | upper limb & shoulder | T3 | N0M0 | 4 |
| 477MM | 82 | M | Cau | malignant melanoma, NOS | scalp & neck          | T2 | N0M0 | 3 |
| 478MM | 78 | F | Cau | epithelioid melanoma    | face                  | T3 | N0M0 | 4 |
| 480MM | 59 | M | Cau | malignant melanoma, NOS | upper limb & shoulder | T2 | N0M0 | 3 |
| 481MM | 85 | M | Cau | malignant melanoma, NOS | upper limb & shoulder | T3 | N0M0 | 4 |
| 482MM | 66 | M | Cau | epithelioid melanoma    | face                  | T3 | N0M0 | 4 |
| 483MM | 85 | F | Cau | epithelioid melanoma    | trunk                 | T4 | N0M0 | 5 |
| 484MM | 70 | F | Cau | malignant melanoma, NOS | upper limb & shoulder | T1 | N0M0 | 3 |
| 511MM | 69 | M | Cau | epithelioid melanoma    | skin, NOS             | T3 | N1M0 | 4 |
| 512MM | 45 | M | Cau | epithelioid melanoma    | trunk                 | T4 | N0M0 | 3 |

Table 7

| Characteristics         | Melanoma (%)  | Nevi (%)      | Normal skin (%) |
|-------------------------|---------------|---------------|-----------------|
| Mean Age                | 65.51 ± 14.55 | 33.17 ± 15.60 | n/a             |
| Gender                  |               |               |                 |
| Female                  | 22 (48.9)     | 9 (50)        | 6               |
| Male                    | 23 (51.1)     | 0 (50)        | 1               |
| Anatomical location     |               |               |                 |
| Face                    | 5 (11.1)      | 3 (17)        |                 |
| Scalp and neck          | 4 (8.0)       | 1 (6)         |                 |
| Trunk                   | 9 (20)        | 10 (55)       | 6 (86)          |
| Upper limb and shoulder | 17 (37.8)     |               |                 |
| Lower limb and hip      | 6 (13.3)      | 4 (22)        |                 |
| Skin, NOS               | 4 (8.9)       |               | 1 (14)          |
| Histological diagnosis  |               |               |                 |
| Epithelioid cell        | 20 (44.4)     |               |                 |
| Spindle cell            | 7 (15.6)      |               |                 |

|                        |           |        |         |
|------------------------|-----------|--------|---------|
| Malignant melanoma NOS | 18 (40)   |        |         |
| Compound nevus         |           | 8 (44) |         |
| Intradermal nevus      |           | 5 (28) |         |
| Atypical nevus         |           | 2 (11) |         |
| Benign nevus, NOS      |           | 3 (17) |         |
| Normal skin            |           |        | 7 (100) |
| T stage (thickness)    |           |        |         |
| T1                     | 11 (24.4) |        |         |
| T2                     | 14 (31.1) |        |         |
| T3                     | 16 (35.6) |        |         |
| T4                     | 4 (8.9)   |        |         |
| N stage                |           |        |         |
| N0                     | 42 (93.3) |        |         |
| N1                     | 3 (6.7)   |        |         |
| M stage                |           |        |         |
| M0                     | 44 (97.8) |        |         |
| M1                     | 1 (2.2)   |        |         |
| Clark level            |           |        |         |
| 2                      | 9 (20)    |        |         |
| 3                      | 16 (35/6) |        |         |
| 4                      | 12 (26.7) |        |         |
| 5                      | 2 (4.4)   |        |         |
| n/a                    | 6 (13.3)  |        |         |

## Example 2

### RNA Isolation from malignant melanoma and benign skin nevi samples

- Qiagen RNeasy™ Mini Kit (QIAGEN Inc., Valencia, CA) was used, with a
- 5 modified protocol to minimize the residual melanin in the RNA sample. For melanocyte containing tissues, four replicate tissue samples derived from individual patient each weighed approximately 5 mg and were used and processed separately. Tissue samples were homogenized in 1.0 ml RLT buffer (QIAGEN) containing 10 µl β-mercaptoethanol (Sigma Chemical Co., St. Louis, MO) by a mechanical
- 10 homogenizer (UltraTurrex T8, IKA-Werke, Staufen, Germany). After homogenization, samples were loaded onto QIAGEN RNeasy™ columns and followed by centrifugation. After discarding the flow-through, 700 µl of RW1 buffer was added; the column was kept for 5 min at room temperature and then centrifuged. This step was repeated 3 times. Then the standard QIAGEN RNeasy™ Mini Kit
- 15 protocol was followed. To remove RNA from the silica gel membrane, a two-step elution was performed. The total RNA derived from the same individual patient tissue was pooled and used for further analysis.

Standard Trizol protocol was used for RNA isolation from tissues that do not contain a significant proportion of melanocytes. Tissue was homogenized in Trizol reagent (Invitrogen, Carlsbad, CA). After centrifugation the top liquid phase was collected and total RNA was precipitated with isopropyl alcohol at -20°C. RNA pellets were washed with 75% ethanol, resolved in water and stored at -80°C until use. RNA quality was examined with an Agilent 2100 Bioanalyzer RNA 6000 Nano Assay (Agilent Technologies, Palo Alto, CA).

Labeled cRNA was prepared and hybridized with the high-density oligonucleotide array HuI 33A Gene Chip (Affymetrix, Santa Clara, CA) containing a total of 22,000 probe sets according to the standard manufacturer protocol. Arrays were scanned using Affymetrix protocols and scanners. For subsequent analysis, each probe set was considered as a separate gene. Expression values for each gene were calculated by using Affymetrix Gene Chip analysis software MAS 5.0. All chips met three quality control standards: "present" call was greater than 35%, scale factor was smaller than 12 when scaled to a target intensity of 600, and background level was less than 150. Lower than usual percent of "present" calls cut-off was chosen because it is difficult to isolate RNA from skin cells (Hipfel et al. (1998)) resulting in lower overall gene expression levels.

### **Example 3**

#### **Data Analysis**

Gene expression data were filtered to include only genes called "present" in 2 or more samples. This filter was used to remove genes that did not change expression in the samples. Of the 22,000 genes presented on the array, 15,795 passed this filter and were used for hierarchical clustering. Prior to clustering, each gene expression signal was divided by the median expression in all samples in the data set. This standardization step minimized the effect of the magnitude of gene expression and group together genes with similar expression patterns in the clustering analysis. Average linkage hierarchical clustering using Pearson correlation was performed on both the genes and the samples using GeneSpring 6.1.

In order to identify differentially expressed genes, we compared the melanoma samples to the benign nevi and the normal skin samples separately. The first analysis

consisted of the 45 melanoma and 7 normal skin samples; the second analysis consisted of 45 melanoma and 18 nevi samples. These two datasets were analyzed separately in following procedures as shown in Fig. 1. Significance analysis of microarray (SAM; Tusher et al. (2001)) and Student T-test were used in gene selection. Parameters for SAM were set as  $\Delta=2.5$  and fold change = 2.0 with 1,000 permutations. FDR was 1%. There were no missing data and the default random number was used. Next percentile analysis was conducted. For up-regulated genes the 30%ile in melanoma samples was compared to the maximum of the normal samples, or that of nevi samples. Student T-test with Bonferroni correction was also performed with cut-off  $p<0.05$  in order to ensure that the selected genes had significant differential expression between the two groups of the samples. As a final step, we identified common genes between the melanoma/benign and melanoma/normal gene lists resulting in the single list of genes upregulated in melanoma shown in Figure 1 where the 439 common genes correspond to SEQ ID NOs: 29-467 as described in Table 15 with the results shown in Table 8.

| PSID        | Median Expression in Melanoma | Fold Change (Cancer vs Benign) | Fold Change (Cancer vs skin) |
|-------------|-------------------------------|--------------------------------|------------------------------|
| 200078 s at | 3954                          | 2                              | 3                            |
| 200601 at   | 9254                          | 2                              | 7                            |
| 200612 s at | 2396                          | 2                              | 5                            |
| 200644 at   | 7240                          | 3                              | 6                            |
| 200660 at   | 14659                         | 3                              | 4                            |
| 200707 at   | 3153                          | 2                              | 51                           |
| 200736 s at | 7305                          | 3                              | 3                            |
| 200737 at   | 2423                          | 2                              | 2                            |
| 200783 s at | 1028                          | 2                              | 2                            |
| 200825 s at | 3746                          | 3                              | 3                            |
| 200827 at   | 1593                          | 2                              | 2                            |
| 200837 at   | 5817                          | 2                              | 5                            |
| 200838 at   | 19225                         | 8                              | 17                           |
| 200839 s at | 28353                         | 5                              | 7                            |
| 200859 x at | 9665                          | 3                              | 7                            |
| 200910 at   | 7780                          | 2                              | 4                            |
| 200950 at   | 8419                          | 3                              | 7                            |
| 200954 at   | 3132                          | 2                              | 6                            |
| 200966 x at | 27388                         | 2                              | 3                            |
| 200967 at   | 6154                          | 2                              | 4                            |
| 200968 s at | 5587                          | 2                              | 7                            |



|             |       |    |    |
|-------------|-------|----|----|
| 200972 at   | 8943  | 3  | 3  |
| 201038 s at | 1480  | 2  | 2  |
| 201051 at   | 5439  | 2  | 5  |
| 201105 at   | 33285 | 4  | 5  |
| 201106 at   | 7546  | 2  | 4  |
| 201188 s at | 1730  | 2  | 8  |
| 201189 s at | 3870  | 3  | 3  |
| 201195 s at | 6005  | 4  | 18 |
| 201202 at   | 1860  | 3  | 2  |
| 201251 at   | 23965 | 5  | 11 |
| 201252 at   | 901   | 2  | 3  |
| 201271 s at | 1648  | 2  | 5  |
| 201291 s at | 601   | 3  | 42 |
| 201313 at   | 2414  | 5  | 6  |
| 201346 at   | 3359  | 2  | 2  |
| 201393 s at | 2166  | 2  | 5  |
| 201416 at   | 4845  | 4  | 4  |
| 201417 at   | 2905  | 2  | 4  |
| 201470 at   | 18525 | 4  | 4  |
| 201474 s at | 2471  | 5  | 22 |
| 201485 s at | 1398  | 2  | 10 |
| 201486 at   | 1105  | 2  | 3  |
| 201536 at   | 2441  | 2  | 4  |
| 201614 s at | 651   | 2  | 4  |
| 201660 at   | 4713  | 7  | 3  |
| 201661 s at | 3336  | 6  | 5  |
| 201662 s at | 2693  | 5  | 3  |
| 201670 s at | 3047  | 2  | 12 |
| 201714 at   | 993   | 2  | 3  |
| 201765 s at | 5475  | 4  | 5  |
| 201792 at   | 3897  | 3  | 4  |
| 201804 x at | 5609  | 3  | 3  |
| 201819 at   | 2575  | 5  | 3  |
| 201850 at   | 11103 | 10 | 20 |
| 201880 at   | 1833  | 2  | 3  |
| 201910 at   | 3854  | 4  | 4  |
| 201911 s at | 2154  | 3  | 4  |
| 201931 at   | 2578  | 3  | 2  |
| 201954 at   | 25901 | 9  | 15 |
| 201976 s at | 7697  | 2  | 6  |
| 202069 s at | 855   | 2  | 3  |
| 202070 s at | 2490  | 4  | 5  |
| 202111 at   | 1380  | 3  | 9  |
| 202154 x at | 10260 | 3  | 4  |
| 202185 at   | 8493  | 4  | 6  |
| 202188 at   | 1269  | 3  | 9  |
| 202219 at   | 5630  | 3  | 7  |

|             |       |    |    |
|-------------|-------|----|----|
| 202224 at   | 2650  | 3  | 4  |
| 202225 at   | 1534  | 2  | 2  |
| 202260 s at | 7877  | 6  | 6  |
| 202295 s at | 15129 | 6  | 4  |
| 202329 at   | 3438  | 4  | 4  |
| 202367 at   | 898   | 2  | 2  |
| 202370 s at | 4669  | 3  | 2  |
| 202478 at   | 7922  | 3  | 11 |
| 202503 s at | 2424  | 3  | 5  |
| 202589 at   | 3494  | 2  | 6  |
| 202603 at   | 3772  | 3  | 2  |
| 202705 at   | 1058  | 4  | 4  |
| 202737 s at | 2865  | 3  | 4  |
| 202779 s at | 3400  | 9  | 55 |
| 202785 at   | 1134  | 2  | 6  |
| 202862 at   | 2540  | 6  | 4  |
| 202898 at   | 4736  | 4  | 63 |
| 202954 at   | 1357  | 2  | 2  |
| 202958 at   | 2401  | 4  | 3  |
| 202961 s at | 19648 | 4  | 5  |
| 202986 at   | 2052  | 3  | 28 |
| 203011 at   | 1346  | 2  | 2  |
| 203022 at   | 1068  | 2  | 2  |
| 203069 at   | 560   | 15 | 13 |
| 203071 at   | 958   | 19 | 3  |
| 203094 at   | 968   | 2  | 2  |
| 203145 at   | 513   | 2  | 2  |
| 203167 at   | 2523  | 3  | 5  |
| 203217 s at | 6416  | 3  | 5  |
| 203234 at   | 725   | 3  | 3  |
| 203256 at   | 7799  | 5  | 31 |
| 203262 s at | 2482  | 2  | 2  |
| 203300 x at | 5278  | 3  | 16 |
| 203315 at   | 3273  | 2  | 2  |
| 203366 at   | 847   | 2  | 2  |
| 203396 at   | 3946  | 2  | 3  |
| 203452 at   | 579   | 4  | 12 |
| 203456 at   | 1132  | 2  | 3  |
| 203502 at   | 657   | 3  | 3  |
| 203518 at   | 2943  | 3  | 7  |
| 203554 x at | 5056  | 3  | 4  |
| 203557 s at | 839   | 2  | 2  |
| 203570 at   | 2553  | 4  | 15 |
| 203590 at   | 3093  | 3  | 7  |
| 203643 at   | 1380  | 3  | 8  |
| 203663 s at | 9720  | 5  | 3  |
| 203668 at   | 1963  | 2  | 3  |

|             |      |    |     |
|-------------|------|----|-----|
| 203693 s at | 588  | 3  | 3   |
| 203695 s at | 1548 | 6  | 3   |
| 203723 at   | 4087 | 5  | 9   |
| 203729 at   | 9154 | 2  | 14  |
| 203730 s at | 517  | 3  | 3   |
| 203731 s at | 691  | 3  | 2   |
| 203775 at   | 1251 | 4  | 3   |
| 203827 at   | 3380 | 11 | 8   |
| 203878 s at | 2036 | 8  | 5   |
| 204014 at   | 7184 | 10 | 46  |
| 204015 s at | 2207 | 5  | 15  |
| 204033 at   | 2154 | 7  | 7   |
| 204092 s at | 664  | 3  | 3   |
| 204099 at   | 2496 | 6  | 6   |
| 204170 s at | 1362 | 3  | 4   |
| 204197 s at | 2889 | 3  | 4   |
| 204198 s at | 4024 | 3  | 3   |
| 204202 at   | 875  | 3  | 3   |
| 204228 at   | 1299 | 2  | 3   |
| 204244 s at | 538  | 4  | 4   |
| 204247 s at | 689  | 3  | 27  |
| 204252 at   | 5294 | 5  | 7   |
| 204262 s at | 1003 | 4  | 5   |
| 204423 at   | 689  | 4  | 3   |
| 204436 at   | 3113 | 3  | 3   |
| 204458 at   | 908  | 3  | 3   |
| 204467 s at | 1910 | 3  | 6   |
| 204584 at   | 9677 | 21 | 15  |
| 204585 s at | 805  | 13 | 17  |
| 204647 at   | 1693 | 3  | 3   |
| 204654 s at | 3894 | 4  | 25  |
| 204709 s at | 312  | 24 | 16  |
| 204778 x at | 537  | 2  | 4   |
| 204779 s at | 1641 | 4  | 4   |
| 204857 at   | 2736 | 3  | 297 |
| 204932 at   | 241  | 3  | 3   |
| 204973 at   | 1478 | 3  | 7   |
| 204995 at   | 496  | 4  | 6   |
| 205051 s at | 3875 | 4  | 3   |
| 205142 x at | 937  | 2  | 3   |
| 205169 at   | 267  | 2  | 7   |
| 205373 at   | 579  | 10 | 8   |
| 205376 at   | 815  | 2  | 3   |
| 205405 at   | 2302 | 4  | 11  |
| 205447 s at | 567  | 13 | 5   |
| 205458 at   | 3288 | 6  | 8   |
| 205566 at   | 2684 | 7  | 8   |

|             |       |    |     |
|-------------|-------|----|-----|
| 205591 at   | 1190  | 5  | 3   |
| 205681 at   | 1316  | 8  | 12  |
| 205690 s at | 9179  | 8  | 9   |
| 205691 at   | 226   | 7  | 5   |
| 205717 x at | 8127  | 3  | 3   |
| 205813 s at | 430   | 11 | 9   |
| 205937 at   | 301   | 7  | 5   |
| 205945 at   | 772   | 2  | 4   |
| 205996 s at | 909   | 2  | 3   |
| 206128 at   | 364   | 3  | 3   |
| 206307 s at | 534   | 8  | 4   |
| 206332 s at | 4671  | 2  | 2   |
| 206397 x at | 1436  | 6  | 43  |
| 206441 s at | 3681  | 6  | 31  |
| 206462 s at | 9953  | 53 | 24  |
| 206503 x at | 419   | 8  | 9   |
| 206617 s at | 898   | 4  | 19  |
| 206630 at   | 23194 | 3  | 46  |
| 206688 s at | 2989  | 3  | 2   |
| 206696 at   | 6446  | 7  | 191 |
| 206777 s at | 683   | 4  | 7   |
| 206864 s at | 421   | 5  | 5   |
| 206976 s at | 3375  | 4  | 3   |
| 207038 at   | 1986  | 9  | 47  |
| 207060 at   | 497   | 4  | 5   |
| 207144 s at | 593   | 17 | 24  |
| 207163 s at | 3217  | 3  | 10  |
| 207183 at   | 230   | 6  | 6   |
| 207592 s at | 350   | 5  | 14  |
| 207614 s at | 2139  | 2  | 6   |
| 207622 s at | 882   | 2  | 16  |
| 207828 s at | 997   | 3  | 3   |
| 208002 s at | 3142  | 3  | 7   |
| 208089 s at | 1374  | 3  | 6   |
| 208308 s at | 12282 | 4  | 9   |
| 208540 x at | 5257  | 2  | 2   |
| 208644 at   | 2242  | 2  | 3   |
| 208657 s at | 1547  | 2  | 4   |
| 208677 s at | 5414  | 3  | 14  |
| 208696 at   | 7351  | 4  | 3   |
| 208710 s at | 1112  | 3  | 44  |
| 208723 at   | 4402  | 3  | 6   |
| 208744 x at | 1673  | 4  | 49  |
| 208837 at   | 3997  | 2  | 3   |
| 208916 at   | 1630  | 3  | 5   |
| 208928 at   | 1439  | 4  | 7   |
| 208956 x at | 7772  | 3  | 2   |

|             |       |    |    |
|-------------|-------|----|----|
| 208974 x at | 6025  | 2  | 6  |
| 208975 s at | 1085  | 2  | 3  |
| 209015 s at | 1739  | 4  | 4  |
| 209036 s at | 8944  | 3  | 3  |
| 209053 s at | 269   | 7  | 10 |
| 209072 at   | 6299  | 4  | 18 |
| 209079 x at | 12870 | 3  | 3  |
| 209081 s at | 3160  | 3  | 2  |
| 209123 at   | 4686  | 4  | 3  |
| 209132 s at | 4385  | 5  | 12 |
| 209172 s at | 268   | 3  | 3  |
| 209197 at   | 820   | 3  | 3  |
| 209198 s at | 491   | 3  | 3  |
| 209247 s at | 1486  | 2  | 2  |
| 209254 at   | 1384  | 4  | 7  |
| 209255 at   | 4283  | 6  | 8  |
| 209256 s at | 4949  | 8  | 6  |
| 209283 at   | 12529 | 5  | 3  |
| 209345 s at | 1678  | 2  | 2  |
| 209407 s at | 1461  | 2  | 6  |
| 209515 s at | 5827  | 5  | 15 |
| 209773 s at | 1243  | 3  | 5  |
| 209825 s at | 765   | 2  | 3  |
| 209827 s at | 4884  | 7  | 7  |
| 209828 s at | 1146  | 4  | 5  |
| 209848 s at | 32959 | 7  | 74 |
| 209875 s at | 3038  | 21 | 12 |
| 209932 s at | 7126  | 3  | 5  |
| 210052 s at | 1085  | 3  | 6  |
| 210073 at   | 337   | 4  | 6  |
| 210111 s at | 7841  | 5  | 3  |
| 210127 at   | 391   | 2  | 9  |
| 210854 x at | 2100  | 2  | 6  |
| 210926 at   | 574   | 2  | 3  |
| 210948 s at | 396   | 2  | 4  |
| 210951 x at | 2501  | 2  | 13 |
| 211013 x at | 498   | 8  | 13 |
| 211052 s at | 1399  | 3  | 2  |
| 211066 x at | 12431 | 2  | 2  |
| 211373 s at | 2063  | 5  | 6  |
| 211564 s at | 1992  | 3  | 2  |
| 211752 s at | 2183  | 2  | 2  |
| 211759 x at | 5674  | 2  | 3  |
| 211833 s at | 502   | 2  | 28 |
| 212000 at   | 339   | 3  | 14 |
| 212070 at   | 13437 | 2  | 4  |
| 212081 x at | 1457  | 2  | 4  |

|             |       |    |     |
|-------------|-------|----|-----|
| 212119 at   | 4415  | 2  | 5   |
| 212178 s at | 2976  | 3  | 14  |
| 212193 s at | 3646  | 3  | 10  |
| 212247 at   | 1716  | 3  | 3   |
| 212285 s at | 4252  | 2  | 4   |
| 212312 at   | 1234  | 4  | 2   |
| 212338 at   | 1598  | 3  | 4   |
| 212402 at   | 3019  | 3  | 4   |
| 212472 at   | 1987  | 5  | 5   |
| 212473 s at | 3747  | 5  | 4   |
| 212512 s at | 1441  | 3  | 2   |
| 212520 s at | 2188  | 2  | 4   |
| 212552 at   | 2611  | 2  | 2   |
| 212715 s at | 1085  | 3  | 4   |
| 212739 s at | 2736  | 2  | 3   |
| 212744 at   | 959   | 3  | 5   |
| 212745 s at | 376   | 2  | 15  |
| 212793 at   | 3123  | 4  | 5   |
| 212796 s at | 2511  | 2  | 2   |
| 213002 at   | 1439  | 2  | 3   |
| 213007 at   | 968   | 3  | 4   |
| 213008 at   | 1086  | 6  | 10  |
| 213096 at   | 924   | 3  | 3   |
| 213131 at   | 1392  | 3  | 3   |
| 213169 at   | 4028  | 5  | 4   |
| 213215 at   | 1926  | 4  | 3   |
| 213217 at   | 5848  | 10 | 6   |
| 213241 at   | 9479  | 4  | 23  |
| 213274 s at | 18263 | 12 | 26  |
| 213275 x at | 17604 | 7  | 3   |
| 213330 s at | 1233  | 2  | 7   |
| 213333 at   | 1845  | 2  | 3   |
| 213338 at   | 932   | 6  | 4   |
| 213392 at   | 1022  | 2  | 2   |
| 213474 at   | 723   | 2  | 3   |
| 213496 at   | 2322  | 3  | 7   |
| 213573 at   | 1643  | 2  | 3   |
| 213587 s at | 10416 | 18 | 9   |
| 213638 at   | 1827  | 26 | 102 |
| 213670 x at | 1959  | 2  | 4   |
| 213720 s at | 2248  | 2  | 3   |
| 213746 s at | 4187  | 3  | 14  |
| 213836 s at | 2605  | 8  | 5   |
| 213895 at   | 1279  | 3  | 4   |
| 213960 at   | 11768 | 80 | 29  |
| 214023 x at | 1602  | 7  | 9   |
| 214068 at   | 2148  | 9  | 10  |

|             |       |     |    |
|-------------|-------|-----|----|
| 214104 at   | 814   | 2   | 3  |
| 214201 x at | 746   | 2   | 3  |
| 214581 x at | 510   | 5   | 6  |
| 214614 at   | 542   | 10  | 9  |
| 214632 at   | 358   | 2   | 2  |
| 214656 x at | 2977  | 2   | 2  |
| 214687 x at | 26310 | 2   | 3  |
| 214708 at   | 249   | 2   | 3  |
| 214710 s at | 575   | 2   | 3  |
| 214714 at   | 2366  | 4   | 9  |
| 214717 at   | 471   | 4   | 4  |
| 214752 x at | 6462  | 3   | 4  |
| 214778 at   | 311   | 3   | 27 |
| 214841 at   | 913   | 9   | 11 |
| 214893 x at | 214   | 10  | 9  |
| 214896 at   | 3071  | 8   | 11 |
| 215025 at   | 2365  | 149 | 93 |
| 215115 x at | 12421 | 34  | 15 |
| 215126 at   | 4940  | 8   | 19 |
| 215155 at   | 505   | 6   | 4  |
| 215311 at   | 10093 | 86  | 30 |
| 215812 s at | 1176  | 3   | 13 |
| 215836 s at | 9406  | 3   | 3  |
| 216194 s at | 5011  | 3   | 3  |
| 216973 s at | 1732  | 6   | 4  |
| 217033 x at | 10961 | 21  | 19 |
| 217104 at   | 317   | 6   | 3  |
| 217226 s at | 2191  | 3   | 3  |
| 217297 s at | 838   | 3   | 21 |
| 217377 x at | 12402 | 27  | 18 |
| 217419 x at | 2742  | 3   | 5  |
| 217624 at   | 349   | 21  | 20 |
| 217799 x at | 1724  | 2   | 11 |
| 217827 s at | 4762  | 2   | 2  |
| 217867 x at | 9024  | 3   | 9  |
| 217871 s at | 19519 | 3   | 11 |
| 217891 at   | 1271  | 2   | 3  |
| 218009 s at | 1557  | 4   | 3  |
| 218030 at   | 1316  | 2   | 3  |
| 218074 at   | 3594  | 2   | 4  |
| 218143 s at | 4007  | 3   | 5  |
| 218151 x at | 1384  | 2   | 2  |
| 218152 at   | 1440  | 2   | 3  |
| 218161 s at | 941   | 5   | 5  |
| 218175 at   | 3563  | 3   | 2  |
| 218330 s at | 3853  | 7   | 4  |
| 218349 s at | 588   | 3   | 14 |

|             |       |    |    |
|-------------|-------|----|----|
| 218359 at   | 796   | 3  | 5  |
| 218376 s at | 1931  | 4  | 4  |
| 218447 at   | 2209  | 2  | 3  |
| 218542 at   | 409   | 3  | 6  |
| 218564 at   | 433   | 2  | 4  |
| 218608 at   | 627   | 3  | 4  |
| 218678 at   | 11356 | 14 | 20 |
| 218774 at   | 1061  | 2  | 5  |
| 218786 at   | 732   | 3  | 2  |
| 218824 at   | 1351  | 3  | 5  |
| 218839 at   | 1996  | 38 | 7  |
| 218856 at   | 3199  | 5  | 4  |
| 218888 s at | 906   | 6  | 5  |
| 218931 at   | 911   | 3  | 2  |
| 218952 at   | 2661  | 7  | 7  |
| 218956 s at | 1840  | 4  | 3  |
| 218980 at   | 1627  | 3  | 8  |
| 218996 at   | 1859  | 4  | 6  |
| 219011 at   | 113   | 4  | 3  |
| 219039 at   | 1852  | 2  | 5  |
| 219040 at   | 480   | 3  | 10 |
| 219041 s at | 3435  | 4  | 2  |
| 219051 x at | 1127  | 3  | 8  |
| 219066 at   | 621   | 4  | 2  |
| 219143 s at | 3618  | 9  | 13 |
| 219148 at   | 426   | 2  | 3  |
| 219152 at   | 365   | 13 | 10 |
| 219219 at   | 859   | 2  | 3  |
| 219361 s at | 1033  | 3  | 7  |
| 219372 at   | 376   | 2  | 2  |
| 219408 at   | 421   | 3  | 36 |
| 219478 at   | 5485  | 80 | 18 |
| 219491 at   | 547   | 3  | 12 |
| 219522 at   | 822   | 4  | 3  |
| 219537 x at | 411   | 3  | 6  |
| 219555 s at | 402   | 12 | 21 |
| 219578 s at | 1419  | 17 | 26 |
| 219634 at   | 686   | 3  | 15 |
| 219637 at   | 355   | 3  | 3  |
| 219703 at   | 378   | 3  | 3  |
| 219742 at   | 409   | 3  | 9  |
| 219895 at   | 528   | 6  | 3  |
| 219933 at   | 1399  | 2  | 2  |
| 220116 at   | 748   | 5  | 8  |
| 220155 s at | 5010  | 5  | 6  |
| 220178 at   | 5915  | 9  | 15 |
| 220454 s at | 581   | 2  | 2  |



|             |       |    |     |
|-------------|-------|----|-----|
| 220864 s at | 8416  | 3  | 3   |
| 220948 s at | 11794 | 2  | 3   |
| 220973 s at | 497   | 4  | 3   |
| 220974 x at | 2540  | 2  | 2   |
| 220980 s at | 3598  | 3  | 2   |
| 221059 s at | 2438  | 5  | 3   |
| 221483 s at | 9194  | 3  | 3   |
| 221484 at   | 3834  | 3  | 3   |
| 221538 s at | 3971  | 3  | 3   |
| 221558 s at | 2356  | 3  | 5   |
| 221577 x at | 4897  | 28 | 38  |
| 221641 s at | 1199  | 2  | 4   |
| 221688 s at | 3740  | 2  | 4   |
| 221710 x at | 728   | 2  | 2   |
| 221732 at   | 931   | 3  | 2   |
| 221759 at   | 1261  | 4  | 21  |
| 221797 at   | 430   | 2  | 7   |
| 221799 at   | 1601  | 5  | 3   |
| 221815 at   | 3293  | 17 | 144 |
| 221882 s at | 1144  | 5  | 6   |
| 221902 at   | 2491  | 4  | 4   |
| 221909 at   | 243   | 22 | 17  |
| 221962 s at | 1132  | 2  | 6   |
| 222116 s at | 4208  | 2  | 4   |
| 222153 at   | 445   | 3  | 8   |
| 222155 s at | 1264  | 3  | 10  |
| 222175 s at | 2415  | 3  | 6   |
| 222196 at   | 224   | 3  | 6   |
| 222199 s at | 2152  | 2  | 3   |
| 222206 s at | 383   | 4  | 12  |
| 222212 s at | 3715  | 3  | 4   |
| 222231 s at | 2724  | 3  | 2   |
| 222234 s at | 754   | 4  | 12  |
| 222240 s at | 1331  | 3  | 3   |
| 222294 s at | 2193  | 3  | 5   |
| 32811 at    | 4194  | 2  | 3   |
| 40560 at    | 1629  | 3  | 5   |
| 44783 s at  | 8503  | 14 | 6   |
| 46665 at    | 7835  | 4  | 3   |
| 55093 at    | 3032  | 4  | 4   |
| 63825 at    | 10096 | 14 | 74  |
| 87100 at    | 737   | 6  | 70  |

We selected a short list of genes with at least 10-fold over-expression in melanoma as compared to the benign specimens. The complete array dataset has been submitted to the NCBI/Genbank GEO database (series entry pending).

Hierarchical clustering revealed four distinct clusters (Fig. 2). Two clusters  
 5 consisted of majority of the melanoma samples (43 out of 45); the third cluster  
 included the majority of benign nevi samples (15 out of 18) and the fourth contained  
 all 7 normal skin specimens. Melanoma samples themselves formed two clusters with  
 35 samples in one cluster and 10 samples in the other. Samples that formed the small  
 cluster represented epithelioid melanoma only, visually contained less melanin and  
 10 demonstrated higher expression of PRAME and MIA genes ( $p < 0.05$ ). The few stage  
 III and IV tumors were all grouped in the small cluster. The large cluster showed  
 higher expression of NTRK3 and nestin (NES) ( $p < 0.05$ ). All melanoma and benign  
 nevi samples demonstrated equally high expression of known melanocyte markers  
 such as tyrosinase and MART-I, confirming that there is comparable melanocyte  
 15 content in these samples. Our data indicate that melanoma, benign nevi and normal  
 skin samples have distinct gene expression profiles and can be separated on molecular  
 basis. Selected genes that were highly expressed in melanoma and their associated  
 functional categories are summarized in Table 9.

Table 9

| psid  | Name        | Median<br>expression in<br>melanoma | Median<br>expression in<br>benign/normal | Fold change |
|---|-------------|-------------------------------------|--|-------------|
| <b>Neural system development and function</b> |             |                                     |  |             |
| 215025_at                                     | NTRK3       | 2365.1                              | 19.9                                     | 118.8       |
| 204709_s_at                                   | KNSL5       | 311.8                               | 13.5                                     | 23.1        |
| 204585_s_at                                   | L1CAM var 1 | 805.2                               | 49                                       | 16.4        |
| 218678_at                                     | NES         | 11355.5                             | 703.6                                    | 16.1        |
| 202260_s_at                                   | STXBP1      | 7877.1                              | 1312                                     | 6.0         |
| 204995_at                                     | p35         | 496.3                               | 89.2                                     | 5.6         |
| 208308_s_at                                   | GP1         | 12281.7                             | 2238.2                                   | 5.5         |
| 201340_s_at                                   | ENC1        | 390.8                               | 74.9                                     | 5.2         |
| 209072_at                                     | MBP         | 6299.1                              | 1215.9                                   | 5.2         |
| <b>Cell movements</b>                         |             |                                     |  |             |
| 214614_at                                     | HOXB9       | 541.6                               | 54.4                                     | 10.0        |
| 205447_s_at                                   | MAP3K12     | 566.5                               | 76.5                                     | 7.4         |
| <b>Tissue morphology</b>                      |             |                                     |  |             |
| 206397_x_at                                   | GDF1        | 1436.1                              | 130.1                                    | 11.0        |

|                             |         |         |        |      |
|-----------------------------|---------|---------|--------|------|
| 205458 at                   | MC1R    | 3287.6  | 458.1  | 7.2  |
| <b>Cancer cell invasion</b> |         |         |        |      |
| 213274 s at                 | CSTTB   | 18262.9 | 1261.4 | 14.5 |
| 208677 s at                 | BSG     | 5413.8  | 1088   | 5.0  |
| <b>Cell cycle control</b>   |         |         |        |      |
| 219578 s at                 | CPEB1   | 1418.5  | 75.6   | 18.8 |
| 207144 s at                 | CITED1  | 593.4   | 33.5   | 17.7 |
| 204252 at                   | CDK2    | 5293.7  | 869.3  | 6.1  |
| 211373 s at                 | PSEN2   | 2063.3  | 403.2  | 5.1  |
| <b>Cell death</b>           |         |         |        |      |
| 221577 x at                 | PLAB    | 4896.9  | 173.9  | 28.2 |
| 205681 at                   | BCL2A1  | 1316.4  | 135.1  | 9.7  |
| <b>Unknown</b>              |         |         |        |      |
| 204545 at                   | PEX6    | 379.1   | 23.9   | 15.9 |
| 201850 at                   | CAPG    | 11103.2 | 725.6  | 15.3 |
| 204014 at                   | DUSP4   | 7183.6  | 601.3  | 11.9 |
| 202779 s at                 | E2-EPF  | 3400    | 323.3  | 10.5 |
| 201954 at                   | ARPC1B  | 25900.7 | 2470   | 10.5 |
| 209848 s at                 | me20m   | 32958.9 | 3778.4 | 8.7  |
| 213112 s at                 | SQSTM1  | 260.4   | 33.9   | 7.7  |
| 218952 at                   | SAAS    | 2660.7  | 368.7  | 7.2  |
| 204099 at                   | SMARCD3 | 2496.2  | 428.2  | 5.8  |
| 206999 at                   | IL12RB2 | 354     | 61.3   | 5.8  |
| 201251 at                   | PKM2    | 23964.7 | 4228.2 | 5.7  |
| 202185 at                   | PLOD3   | 8493.2  | 1541.7 | 5.5  |

**Example 4****Identification of genes differentially expressed in melanoma**

A total of 70 gene expression profiles were used for analysis. The median percentages of "present calls" for melanoma, benign and normal sample groups were 43.8%, 46.9% and 41.7%. Sixty microarrays (86%) had scaling factors within 3-fold range of the minimum value. Ten chips with the scaling factors more than 3 were equally distributed between the sample categories, melanoma, benign and normal.

Unsupervised hierarchical clustering result revealed a distinct separation of the melanoma, benign nevi and normal skin samples (Fig. 2). We observed four clusters, including two clusters consisting of majority of the melanoma samples (43 out of 45), the third cluster contained all 7 normal skin, 3 benign nevi and 2 melanoma specimens and the fourth cluster, that included 14 of the 18 benign nevi samples. Source of the samples did not affect clustering. Specimens originated from different sources were clustered together according the sample type (melanoma, benign or

normal). To further test the stability of the clustering patterns, we used an alternative cut-off on gene filtering prior to the cluster analysis. Specifically, we retained genes that have at least 10% "present" calls in each of the melanoma, benign nevi and skin samples. With this cut-off, we obtained 15,306 genes and repeated hierarchical  
5 clustering. The cluster pattern on the patient samples was the same as the one from the 15,795 from the 2 "present" calls, confirming clustering stability.

The single nevi sample that clustered with the melanoma samples is an atypical nevi (moderate degree) sample with no melanoma *in-situ* present. All three nevi samples that clustered with normal skin are compound nevi samples and one of them  
10 has melanocyte content lower than the other nevi specimens. The melanoma samples themselves formed two clusters with 34 samples in the large and 9 samples in the smaller cluster. Samples that formed the small cluster represented epithelioid melanoma only and visually contained less melanin. The few stage III and IV tumors, used in our study, were all grouped in the small cluster. The large cluster was  
15 composed from epithelioid, spindle cell and melanoma of mixed histology specimens with more significant presence of melanin. The large cluster included Stage I and Stage II specimens only.

Distinct gene clusters were found in association to melanoma. This can be characterized by up-regulated (Fig. 2, A, B, C) and down-regulated (Fig. 2, E) genes  
20 in the melanoma samples. At the same time, melanoma and benign nevi samples demonstrated high expression of known melanocyte markers, such as MART-I (Fig. 3, D) confirming a comparable content of melanocyte in these samples and inability of melanocyte specific markers to differentiate them. Our data indicate that melanoma, benign nevi and normal skin samples have distinct gene expression  
25 profiles and can be separated on their molecular basis.

In order to identify genes upregulated in malignant melanoma, we applied SAM in combination with t-test with Bonferroni correction and percentile analysis (Fig. 1). Bonferroni-adjusted t-test and percentile analyses were used to address the multiple testing issue and the heterogeneity of the tumor samples, respectively. As the result  
30 of these analyses, 439 genes were selected and are summarized in Table 15 as SEQ ID NOs: 29-467. Out of 439 genes up-regulated in melanoma, we selected a short list of

33 genes that had more than 10-fold over-expression in the melanoma samples than that of the benign specimens. These include many genes with known association with malignant melanoma such as NTRK3 (Xu et al. (2003)), LICAM (Fogel et al. (2003); and Thies et al. (2002)), me20m (Adema et al. (1994)), as well as novel genes. Genes with more than 10-fold overexpression in melanoma are presented in Table 10.

Table 10

| Psid        | Description          | Median Exp Melanoma | Fold change (Can v Benign) | Fold change (Can v skin) |
|-------------|----------------------|---------------------|----------------------------|--------------------------|
| 215025 at   | NTRK3                | 2365                | 149                        | 93                       |
| 215311 at   | EUROIMAGE 21920      | 10093               | 86                         | 30                       |
| 213960 at   | EUROIMAGE 51358      | 11768               | 80                         | 29                       |
| 219478 at   | WFDC1                | 5485                | 80                         | 18                       |
| 218839 at   | HEY1                 | 1996                | 38                         | 7                        |
| 215115 x at | TEL oncogene         | 12421               | 34                         | 15                       |
| 221577 x at | PLAB                 | 4897                | 28                         | 38                       |
| 217377 x at | ETV6-NTRK3 fusion    | 12402               | 27                         | 18                       |
| 213638 at   | PHACTR1              | 1827                | 26                         | 102                      |
| 204709 s at | KNSL5                | 312                 | 24                         | 16                       |
| 221909 at   | Hyp protein FLJ14627 | 243                 | 22                         | 17                       |
| 204584 at   | L1CAM                | 9677                | 21                         | 15                       |
| 209875 s at | SPP1                 | 3038                | 21                         | 12                       |
| 217624 at   | PDAP1                | 349                 | 21                         | 20                       |
| 203071 at   | SEMA3B               | 958                 | 19                         | 3                        |
| 213587 s at | C7ORF32              | 10416               | 18                         | 9                        |
| 221815 at   | ABHD2                | 3293                | 17                         | 144                      |
| 219578 s at | CPEB1                | 1419                | 17                         | 26                       |
| 207144 s at | CITED1               | 593                 | 17                         | 24                       |
| 203069 at   | SV2A                 | 560                 | 15                         | 13                       |
| 218678 at   | NES                  | 11356               | 14                         | 20                       |
| 219152 at   | PODXL2               | 365                 | 13                         | 10                       |
| 205447 s at | MAP3L12              | 567                 | 13                         | 5                        |
| 213274 s at | CTSB                 | 18263               | 12                         | 26                       |
| 219555 s-at | BMO39                | 402                 | 12                         | 21                       |
| 203827 at   | WIPI49               | 3380                | 11                         | 8                        |
| 205813 s at | MAT1A                | 430                 | 11                         | 9                        |
| 201850 at   | CAPG                 | 11103               | 10                         | 20                       |
| 205373 at   | CTNNA2               | 579                 | 10                         | 8                        |
| 214614 at   | HLXB9                | 542                 | 10                         | 9                        |
| 213217      | ADCY2                | 5848                | 10                         | 6                        |
| 204014 at   | DUSP4                | 7184                | 10                         | 46                       |
| 214893      | HCN2                 | 214                 | 10                         | 9                        |

We further selected three genes over-expressed in melanoma, including NTRK3, PLAB, LICAM, for quantitative real-time RT-PCR validation of the microarray results (Fig. 3). PLAB is a novel gene, whose differential expression in melanoma was not reported before at our best knowledge. For LICAM and NTRK3, differential

expression in melanoma was demonstrated at protein level only. Xu et al. (2003); Fogel et al. (2003); and Thies et al. (2002). Moreover, we identified PLAB and LICAM as the best combination, on complementary basis, to separate melanoma from benign/normal tissues in our study. GPIIO is known as a melanoma specific marker and was selected as positive control. For the RT-PCR assay we used a panel of 14 primary melanoma, 7 benign nevi and 5 normal skin samples, isolated from the same tissues as used for the microarray study. The expression value of each gene was normalized to the housekeeping control gene PBGD. The correlation coefficients between the RT-PCR and the microarray results for LICAM, NTRK3, PLAB and gp100 are 0.79, 0.86, 0.87 and 0.88, respectively. This result indicates that the RT-PCR results are highly consistent with the microarray data.

#### **Example 5**

##### **Pathway Analysis of Differentially Expressed Genes**

Functional analysis of genes differentially expressed in melanoma was performed using Ingenuity™ Pathway Analysis Software Application (Ingenuity, Mountain View, CA). Functional categories or canonical pathways that have p-value of less than 0.05 were selected. Specificity of canonical pathways identification was tested using randomly selected genes.

In order to gain further insight into a potential mechanism that differentiates melanoma from benign and normal tissue, we used Ingenuity pathway analysis software to identify canonical pathways associated with melanoma. The results analysis revealed that many of the genes in amyloid processing were up-regulated in the melanoma samples. To verify specificity of our observation, we selected three random lists of genes from Affymetrix Hu133A microarray and subjected them to Ingenuity pathway analysis. None of these lists produced a significant association to amyloid processing or any other canonical pathways. To confirm the activation of this canonical pathway in melanoma, gene expression data for all the genes in the pathway were retrieved. Fold-change and p-value of differential expression between melanoma and benign/normal tissues were calculated. Out of the 34 genes included in the amyloid processing pathway (Esler et al. (2001); and Giancotti et al. (1999)), 25 demonstrated up-regulation trend and for 19 of them (56%), differential expression was statistically significant (p-value < 0.05; Fig 4). As the additional control, we

randomly selected two metabolic pathways with a similar number of genes. Out of the 63 genes in alanine synthesis pathway, 8 of them (13%) showed significant up-regulation with p-value less than 0.05. Out of the 47 genes in histidine synthesis pathway, only 2 genes (4%) were found using the same criteria. For the first time, our data strongly indicated that activation of the amyloid processing pathway is involved in malignant melanoma.

### Example 6

#### RT-PCR Validation of Microarray Results

Ten microgram total RNA from each sample was treated with DNase I and reverse-transcribed with oligo (dT) primer using Superscript II reverse transcriptase according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). A control gene PBGD was previously tested and reported as a housekeeping gene. Vandesompele et al. (2003). Primers and MGB-probes for me20m (gpl00), LICAM, NTRK3, and the control gene PBGD were designed using Primer Express software (Applied Biosystems, Foster City, CA). The PLAB (MIC1) gene probe was FAM-TAMRA based since sequences were inadequate to design MGB based probes. Primer/probe sequences were as follows:

Table 11

| Description   | Sequence                                 | SEQ ID NO: |
|---------------|--|------------|
| me20m forward | TGTGTCTCTGGCTGATACCAACA                  | 983        |
| me20m reverse | TTCTTGACCAGGCATGATAAGCT                  | 984        |
| me20m probe   | (6-FAM) CTGGCAGTGGTCAGC                  | 985        |
| L1CAM forward | GCTGGGACTGGGAACAGAACT                    | 10         |
| L1CAM reverse | GGAGCAGAGATGGCAAAGAAA                    | 11         |
| L1CAM probe   | (6-FAM) TCCCCACCATCTGCTGT                | 12         |
| NTRK3 forward | GCCCCGGCACCCCTTTA                        | 16         |
| NTRK3 reverse | AACCCTGCCAGTGGTGGAT                      | 17         |
| NTRK3 probe   | (6-FAM) CAGATGGGTGTTTTC                  | 18         |
| PLAB forward  | GGCAGAATCTTCGTCCGCA                      | 4          |
| PLAB reverse  | GGACAGTGGTCCCCGTTG                       | 5          |
| PLAB probe    | (6-FAM) CCCAGCTGGAGTTGCACTTGCGGCC(TAMRA) | 6          |

|              |  |     |
|--------------|--|-----|
| PBGD forward | CTGCTTCGCTGCATCGCTGAAA                           | 986 |
| PBGD reverse | CAGACTCCTCCAGTCAGGTACA                           | 987 |
| PBGD probe   | (6-FAM)<br>CCTGAGGCACCTGGAAGGAGGCTGCAGTGT(TAMRA) | 988 |

All primers and probes were tested for optimal amplification efficiency above 90%. The standard curve was composed of six 10-fold dilutions of target gene PCR product with copy numbers ranging from 10 to 10<sup>6</sup>. RT-PCR amplification was carried out in a 20µl reaction mix containing 50ng template cDNA, 2 x TaqMan® universal PCR master mix (12.5µl) (Applied Biosystems, Foster City, CA), 50nM forward and reverse primers, and 250nM probe. Reactions were run on an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). The cycling conditions were: 2 min of AmpErase UNG activation at 50°C, 10 min of polymerase activation at 95°C and 50 cycles at 95°C for 15 sec and annealing temperature (60°C) for 60 sec. In each assay, a standard curve and a no-template control along with template cDNA were included in duplicate for both the gene of interest and the control gene. The relative quantity of each target gene was represented as  $\Delta C_t$ , which is equal to  $C_t$  of the target gene subtracted by  $C_t$  of the control gene.

To confirm the melanoma specific genes identified by the microarray analysis, four genes (LICAM, NTRK3, PLAB and gp100) were selected for quantitative real-time RT-PCR validation (Fig. 4). The expression value of each gene was normalized to housekeeping control PBGD. The correlation coefficient between the RT-PCR and the microarray results for LICAM, NTRK3, PLAB and gp100 are 0.79, 0.86, 0.87 and 0.88, respectively, indicating that the RT-PCR results are highly consistent with the microarray data.

#### Example 7

##### One-step qRT-PCR Assays Using RNA-specific Primers and Cutoff Establishment

Evaluation of expression of selected genes was carried out with one-step RT-PCR with RNA from primary melanoma, benign nevi, normal skin, melanoma LN metastasis and melanoma-free lymph nodes. Beta-actin was used as a housekeeping gene to control for the input quantity and quality of RNA in the reactions. DNase



treatment was not used. Instead, primers or probes were designed to span an intron so they would not report on genomic DNA. Eight ng of total RNA was used for the RT-PCR. The Total RNA was reverse transcribed using 40X Multiscribe and RNase inhibitor mix contained in the TaqMan® One Step PCR Master Mix Reagents Kit

5 (Applied Biosystems, Foster City, CA). The cDNA was then subjected to the 2x Master Mix without UNG and PCR amplification was carried out on the ABI 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) in the 384-well block format using a 10 µl reaction size. The primer and probe concentrations were 4 µM and 2.5 µM, respectively. The reaction mixture was incubated at 48°C for 30 min

10 for the reverse transcription, followed by a Amplitaq activation step of 95°C for 10 min and finally 40 cycles of 95°C for 15 sec denaturing and 60°C for 1 min anneal and extension. On each plate a standard curve is generated from 8 pg to 80 ng and when the R2 value was greater than 0.99 the Cycle Threshold (Ct) values were accepted.

15 Sequences used in the reactions were as follows, each written in the 5' to 3' direction.

Table 12

| Description        | Sequence                      | SEQ ID NO: |
|--------------------|-------------------------------|------------|
| L1CAM Forward      | CCACAGATGACATCAGCCTCAA        | 13         |
| L1CAM Reverse      | GGTCACACCCAGCTCTTCCTT         | 14         |
| L1CAM probe        | TGGCAAGCCCGAAGTGCAGTTCC       | 15         |
| Tyrosinase Forward | CTTTAGAAATACACTGGAAGGATTTGCTA | 1000       |
| Tyrosinase Reverse | CATTGTGCATGCTGCTTTGA          | 1001       |
| Tyrosinase probe   | TCCACTTACTGGGATAGCGGATGCCTC   | 1002       |
| MART1 Forward      | ACTTCATCTATGGTTACCCCAAGAA     | 1003       |
| MART1 Reverse      | TCCCAGCGGCCTCTTCA             | 1004       |
| MART1 Probe        | CACGGCCACTCTTACACCACGGC       | 1005       |
| HMB45 Forward      | CTTAAGGCTGGTGAAGAGACAAGTC     | 1006       |
| gp100 Reverse      | CAGGATCTCGGCACTTTCAATAC       | 1007       |
| gp100 Probe        | TCGATATGGTTCCTTTTCCGTCACCCTG  | 1008       |
| PLAB Forward       | ATTCGAACACCGACCTCGTC          | 1009       |

|              |                           |      |
|--------------|---------------------------|------|
| PLAB Reverse | CGCAGGTGCAGGTGGC          | 1010 |
| PLAB Probe   | GATACTCACGCCAGAAAGTGCGGCT | 1011 |

For each sample  $\Delta Ct = Ct(\text{Target Gene}) - Ct \beta\text{-actin}$  was calculated.  $\Delta Ct$  has been widely used in clinical RT-PCR assays and was chosen as a straightforward method. Cronin et al. (2004). T-test was performed on  $\Delta Ct$  between the melanoma and non-melanoma samples including both primary and LN samples. We then used  $\Delta Ct$  to construct two scores for each patient. One score was derived from a combination of 2 melanoma specific genes, PLAB and LICAM; and the other score was derived from a combination of 3 conventional melanoma markers, tyrosinase, gp100 and MART1. The score was defined as the weighted sum of  $\Delta Ct$  values of the tested genes with the corresponding  $t$  statistics as the weight. The two scores were normalized to have the same mean in order to compare them on the same scale.

We examined a combination of two highly overexpressed in melanoma genes, PLAB and LICAM, in a variety of clinical tissue samples containing malignant melanocytes (primary melanoma and melanoma LN metastasis), benign melanocytes (benign skin nevi) and normal samples (normal skin and melanoma-free LN) by RT-PCR. The primary tissues were the same as those used for the microarray study while all the LN specimens were derived from independent patients. Conventional melanoma markers, such as tyrosinase, gp100 and MART1, were also tested on the same samples as the controls because they are the most commonly used markers for the melanoma molecular assays in current clinical studies. Riboldi et al. (2003); Abrahamsen et al. (2005); and Kammula et al. (2004). Calculated scores were presented on Fig. 4A for PLAB and LICAM and on Fig. 4B for tyrosinase, gp100 and MART1. The results demonstrated significant difference in expression of PLAB and LICAM between malignant melanoma samples (primary and LN metastasis) and benign nevi and normal LN. In contrast, three conventional markers showed similar expression levels in benign and melanoma samples. To further demonstrate the ability of gene markers to separate benign and malignant tissues, we tested two cut-offs; first was set up as the highest score in primary normal samples and the second as the highest score in benign nevi samples. For each cut-off we estimated sensitivity and of the assay in the LN samples. With the cut-off determined on the normal

samples, the new markers and the conventional markers gave sensitivity of 90% and 83%, respectively. Using the cut-off determined on the benign samples, the sensitivity for the new and conventional markers were 88% and 42%. The results indicated that the new markers potentially have better abilities to differentiate tissues containing benign and malignant melanocytes.

### Example 8

#### Multiplex Assay

##### Materials and methods

Each reaction was set up in a final volume of 25  $\mu$ l containing the following:

|    |                     |           |
|----|---------------------|-----------|
| 10 | forward primer      | 400 nM    |
|    | reverse primer      | 500 nM    |
|    | PLAB probe          | 150 nM    |
|    | Tyrosinase probe    | 300 nM    |
|    | LICAM probe         | 200 nM    |
| 15 | PBGD probe          | 200 nM    |
|    | Tth                 | 5 U       |
|    | Ab TP 6-25          | 1 $\mu$ g |
|    | Glycerol            | 10%       |
|    | Tris-HCl            | 3J mM     |
| 20 | NaCl                | 4 mM      |
|    | EDTA                | 0.004 mM  |
|    | Tween-20            | 0.22%     |
|    | NP-40               | 0.02%     |
|    | DTT                 | 0.04 mM   |
| 25 | Potassium Hydroxide | 20.5 mM   |
|    | Bicine              | 50 mM     |
|    | Potassium Acetate   | 115 mM    |
|    | Albumin, bovine     | 5 $\mu$ g |
|    | Trehalose           | 0.15 M    |
| 30 | dNTP                | 0.2 mM ea |
|    | MgCl <sub>2</sub>   | 0.5 mM    |
|    | MnSO <sub>4</sub>   | 3.5 mM    |
|    | Primers             | 300 nM ea |
|    | Probes              | 200 nM ea |

35 The primer and probe sequences are provided in Table 13.

Table 13

| SEQ ID NO | Sequence 5'-3'                          | Function           |
|-----------|---|--------------------|
| 43        | gaacaccgacctcgtccc                      | PLAB Upper Primer  |
| 44        | ggcggcccagagata                         | PLAB Lower Primer  |
| 45        | Fam-cgccagaagtgcggctgggat-BHQ1-tt       | PLAB Probe         |
| 55        | actcagcccagcatcattcttc                  | Tyr Upper Primer   |
| 56        | atggctgtgtactcctccaatc                  | Tyr Lower Primer   |
| 57        | Q570-cttcctcttggcagattgtctgtacg BHQ2-tt | Tyr Probe          |
| 49        | ccacagatgacatcagcctcaa                  | L1CAM Upper Primer |
| 50        | ggtcacacccagctcttctt                    | L1CAM Lower Primer |
| 51        | CalRed-tggcaagcccgaagtgcagttcc-BHQ2-tt  | L1CAM Probe        |
| 58        | ccacacacagcctactttcaa                   | PBGD Upper Primer  |
| 59        | taccacgcgaatcactctca                    | PBGD Lower Primer  |
| 60        | Q670-aacggcaatgcggctgcaacggcgga-BHQ2-tt | PBGD Probe         |

The reactions are run with PLAB in Fam, Tyrosinase in Cy3, L1CAM in Texas Red, and PBGD in Cy5 channels. The cycling protocol used is described below and takes 30 min to complete.

- 5 95°C x 15 sec  
65°C x 420 sec  
40°cycles of:  
95°C for 5 sec  
62°C for 15 sec - fluor read
- 10 The thresholds used are 30 in Fam, 20 in Cy3, 20 in Texas Red, and 20 in Cy5 channels. The thresholds employed in the Cy3 and Texas red channels can be lowered. The results obtained are summarized in Table 14.

Table 14  
Best Marker Combinations

| Markers                    | % Sensitivity (95% CI) | % Specificity (95% CI) |
|----------------------------|------------------------|------------------------|
| L1CAM + PLAB               | 82 (73-89)             | 96 (87-100)            |
| Tyrosinase + ME20M (GP100) | 63 (52-72)             | 100 (94-100)           |
| L1CAM + PLAB + Tyrosinase  | 87 (79-93)             | 96 (87-100)            |

- 15 Ct cutoffs:  
L1CAM 27  
PLAB 29  
Tyrosinase 23  
ME20M (GP100) 23.5

- 20 Note: these data are benchmarked against H&E pathology only. The amplification efficiency in each of the 4 reactions is high and the reaction is also linear over 5 logs (as judged by the R2 value which is >0.99 in all cases). Therefore,

these data demonstrate a working 4 plex, rapid assay. These data suggest that PLAB is the primary marker and complementation, achieved with LICAM, further increases sensitivity. If required, addition of tyrosinase as a third marker further complements LICAM and PLAB and increases sensitivity. Tyrosinase can be dropped from the assay, if needed, without affecting the performance of the remaining markers.

### Discussion

We performed gene expression profiling analysis of primary melanoma, benign nevi and normal skin tissue specimens in order to find melanoma specific gene markers for potential use in the LN molecular staging assay. Novel genes that are highly and differentially expressed in malignant melanoma samples were identified. Inclusion of benign nevi in the experimental design was key to our study. In contrast to normal skin, melanocyte content in benign nevi is close to that in melanoma. This was confirmed, in addition to histological assessment, by equally high expression level of conventional melanoma markers such as tyrosinase and MART1 in both melanoma and nevi tissue specimens. Similar cellular composition allowed us to monitor gene expression changes specifically associated with melanocyte malignant transformation, not just with melanocyte lineage differentiation. As the result, we identified novel genes specifically overexpressed in melanoma. One of the novel highly overexpressed in melanoma genes, prostate differentiation factor (PLAB, MIC1), is a member of transforming growth factor-beta superfamily and also known to be associated with other malignancies. Bae et al. (2003); and Welsh et al. (2003). PLAB reduces cell adhesion (Yamauchi et al. (2003)), implicating its potential role in melanoma progression. Pathway analysis of the overexpressed genes in melanoma indicated that many of these genes belong to neural tissue functioning and development, suggesting that dedifferentiation of melanocytes and activation of the processes related to a pluripotent progenitor cell might be important for melanoma development and progression. Moreover, the analysis of canonical pathways showed that neural tissue associated amyloid processing is significantly modulated in melanoma. Amyloid processing (APP) pathway itself has not been associated with melanoma development and progression before. Many genes in the APP pathway, such as members of the  $\beta$ - and  $\gamma$ -secretase family (BACE2, PSEN2) also participate

in the Notch pathway and play a role of cleavage of integral membrane proteins in both Notch and APP. Esler et al. (2001). Notch suppresses differentiation and helps maintain neural crest stem cells in undifferentiated state (Gangemi et al. (2004)) and Notch's involvement in melanoma and, particularly, the role of Y-secretases is the  
5 focus of many studies. Hoek et al. (2004); Baldi et al. (2003); and Wilson et al. (2000).

We have compared our results to the recent study of Haqq et al (2005). In their work, cDNA microarray containing 20,862 probes was used to profile benign nevi, primary melanoma and metastatic melanoma specimens. The sample set included  
10 metastatic and primary melanoma and benign nevi. Similar clustering results that separated the benign nevi and the primary malignant melanoma tissues were found in their study. Common genes were reported in both studies that can discriminate melanoma from benign nevi including kinesin-like 5 (KNSL5), prostate differentiation factor (PLAB), CITED1, osteopontin (SPPI), cathepsin B (CSTB),  
15 cadherin 3 (CDH3), presenilin 2 (PSEN2).

Our results of the one-step RT-PCR assay demonstrated that novel melanoma specific gene PLAB and LICAM expressed not only in primary melanoma tissues but also in melanoma LN metastasis. Moreover, the ability to differentiate malignant melanoma from benign nevi made them better candidates than the conventional  
20 markers for the molecular test of melanoma diagnostics. With further validation in clinical studies, these genes could be developed as specific markers for a molecular staging assay to detect melanoma micrometastasis during sentinel lymph node (SLN) biopsy procedure. Another potential application of the genes is for diagnosis of melanocyte lesions with uncertain pathological features.

25 Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention.

Table 15 Sequence Descriptions, names and SEQ ID NOs:

|    |             |           |           |  |
|----|-------------|-----------|-----------|--|
| 1  |             |           | PLAB      |  |
| 2  |             |           | L1CAM     |  |
| 3  |             |           | NTRK      |  |
| 4  |             |           |           | PLAB forward primer                                      |
| 5  |             |           |           | PLAB reverse primer                                      |
| 6  |             |           |           | PLAB probe   |
| 7  |             |           |           | PLAB upper primer  |
| 8  |             |           |           | PLAB lower primer  |
| 9  |             |           |           | PLAB probe   |
| 10 |             |           |           | L1CAM forward primer                                     |
| 11 |             |           |           | L1CAM reverse primer                                     |
| 12 |             |           |           | L1CAM probe  |
| 13 |             |           |           | L1CAM upper primer                                       |
| 14 |             |           |           | L1CAM lower primer                                       |
| 15 |             |           |           | L1CAM probe  |
| 16 |             |           |           | NTRK primer  |
| 17 |             |           |           | NTRK primer  |
| 18 |             |           |           | NTRK probe   |
| 19 |             |           |           | Tyrosinase upper primer                                  |
| 20 |             |           |           | Tyrosinase lower primer                                  |
| 21 |             |           |           | Tyrosinase probe   |
| 22 |             |           |           | PBGD upper primer  |
| 23 |             |           |           | PBGD lower primer  |
| 24 |             |           |           | PBGD probe   |
| 25 |             |           |           | PLAB amplicon  |
| 26 |             |           |           | L1CAM amplicon   |
| 27 |             |           |           | Tyrosinase amplicon                                      |
| 28 |             |           |           | PBGD amplicon  |
| 29 | 200078_s_at | BC005876  |           | ATPase, H <sup>+</sup> transporting, lysosomal           |
| 30 | 200601_at   | U48734    |           | non-muscle alpha-actinin                                 |
| 31 | 200612_s_at | NM_001282 | AP2B1     | adaptor-related protein complex 2, $\beta$ 1             |
| 32 | 200644_at   | NM_023009 | MACMARCKS | macrophage myristoylated alanine-rich C kinase substrate |
| 33 | 200660_at   | NM_005620 | S100A11   | S100 calcium-binding protein A11                         |
| 34 | 200707_at   | NM_002743 | PRKCSH    | protein kinase C substrate 80K-H                         |
| 35 | 200736_s_at | NM_000581 | GPX1      | glutathione peroxidase 1                                 |
| 36 | 200737_at   | NM_000291 | PGK1      | phosphoglycerate kinase 1                                |
| 37 | 200783_s_at | NM_005563 | LAP18     | leukemia-assoc phosphoprotein p18                        |
| 38 | 200825_s_at | NM_006389 | ORP150    | oxygen regulated protein (150kD)                         |
| 39 | 200827_at   | NM_000302 | PLOD      | procollagen-lysine, 2-oxoglutarate 5-dioxygenase         |
| 40 | 200837_at   | NM_005745 | DXS1357E  | accessory proteins BAP31BAP29                            |
| 41 | 200838_at   | NM_001908 | CTSB      | cathepsin B  |
| 42 | 200839_s_at | NM_001908 | CTSB      |  |
| 43 | 200859_x_at | NM_001456 | FLNA      | filamin A, alpha   |
| 44 | 200910_at   | NM_005998 | CCT3      | chaperonin containing TCP1, sub 3( $\gamma$ )            |
| 45 | 200950_at   | NM_006409 | ARPC1A    | actin related protein 23 complex, sub 1A                 |
| 46 | 200950_at   | NM_006409 | ARPC1A    |  |
| 47 | 200966_x_at | NM_000034 | ALDOA     | aldolase A, fructose-bisphosphate                        |
| 48 | 200967_at   | NM_000942 | PPiB      | peptidylprolyl isomerase B                               |
| 49 | 200968_s_at | NM_000942 | PPiB      |  |
| 50 | 200972_at   | BC000704  |           | tetraspan 3  |

|    |             |           |              |   |
|----|-------------|-----------|--------------|---|
| 51 | 201038_s_at | BE560202  |              | putative HLA class II assoc protein I   |
| 52 | 201051_at   | BE560202  |              | putative HLA class II assoc protein I   |
| 53 | 201105_at   | NM_002305 | LGALS1       | lectin, galactoside-binding, soluble, 1   |
| 54 | 201106_at   | NM_002085 | GPX4         | glutathione peroxidase 4  |
| 55 | 201188_s_at | D26351    | ITPR3        | type 3 inositol 1,4,5-trisphosphate receptor  |
| 56 | 201189_s_at | NM_002224 | ITPR3        |   |
| 57 | 201195_s_at | AB018009  |              | L-type amino acid transporter 1   |
| 58 | 201202_at   | NM_002592 | PCNA         | proliferating cell nuclear antigen  |
| 59 | 201251_at   | NM_002654 | PKM2         | pyruvate kinase, muscle   |
| 60 | 201252_at   | NM_006503 | PSMC4        | proteasome 26S subunit, ATPase, 4   |
| 61 | 201271_s_at | NM_016732 | RALY         | RNA-binding protein transcript var 1  |
| 62 | 201291_s_at | NM_001067 |              | topoisomerase (DNA) II alpha  |
| 63 | 201313_at   | NM_001975 | ENO2         | enolase 2   |
| 64 | 201346_at   | NM_024551 | FLJ21432     | hypothetical protein FLJ21432   |
| 65 | 201393_s_at | NM_000876 | IGF2R        | insulin-like growth factor 2 receptor   |
| 66 | 201416_at   | NM_003107 |              | SRY-box 4   |
| 67 | 201417_at   | NM_003107 |              | SRY-box 4   |
| 68 | 201470_at   | NM_004832 | GSTTLp28     | glutathione-S-transferase like;<br>glutathione transferase omega                    |
| 69 | 201474_s_at | NM_002204 | ITGA3        | integrin, alpha 3 transcript variant a  |
| 70 | 201485_s_at | BC004892  | RCN2         | reticulocalbin 2, EF-hand calcium binding domain                                    |
| 71 | 201486_at   | NM_002902 | RCN2         |   |
| 72 | 201536_at   | AL048503  |              | DKFZp586M1524   |
| 73 | 201614_s_at | NM_003707 | RUVBL1       | RuvB (E coli homolog)-like 1  |
| 74 | 201660_at   |           | FACL3        | fatty-acid-Coenzyme A ligase, long-chain 3  |
| 75 | 201661_s_at | NM_004457 | FACL3        |   |
| 76 | 201662_s_at | D89053    |              | Acyl-CoA synthetase 3   |
| 77 | 201670_s_at | M68956    | MARCKS 80K-L | myristoylated alanine-rich C-kinase substrate                                       |
| 78 | 201714_at   | NM_001070 | TUBG1        | tubulin, gamma 1  |
| 79 | 201765_s_at | AL523158  |              | hexosaminidase A  |
| 80 | 201792_at   | NM_001129 | AEBP1        | AE-binding protein 1  |
| 81 | 201804_x_at | NM_001281 | CKAP1        | cytoskeleton-associated protein 1   |
| 82 | 201819_at   | NM_005505 | CD36L1       | CD36 antigen-like 1   |
| 83 | 201850_at   | NM_001747 | CAPG         | capping protein gelsolin-like   |
| 84 | 201880_at   | NM_005744 |              | ariadne (Drosophila) homolog,<br>ubiquitin-conjugating enzyme E2-binding protein, 1 |
| 85 | 201910_at   | BF213279  | FARP1        | RhoGEF & pleckstrin domain 1  |
| 86 | 201911_s_at | NM_005766 | FARP1        |   |
| 87 | 201931_at   | NM_000126 | ETFA         | electron-transfer-flavoprotein, alpha polypeptide                                   |
| 88 | 201954_at   | NM_005720 | ARPC1B       | actin related protein 23 com, sub 1A  |
| 89 | 201976_s_at | NM_012334 | MYO10        | myosin X  |
| 90 | 202069_s_at | AI826060  | IDH3A        | isocitrate dehydrogenase 3 alpha  |
| 91 | 202070_s_at | NM_005530 | IDH3A        |   |
| 92 | 202111_at   | NM_003040 | SLC4A2       | solute carrier fam 4 anion exchanger mem 2  |
| 93 | 202154_x_at | NM_006086 | TUBB4        | tubulin, beta, 4  |
| 94 | 202185_at   | NM_001084 | PLOD3        | procollagen-lysine, 2-oxoglutarate 5-dioxygenase                                    |



|     |             |           |          |   |
|-----|-------------|-----------|----------|---|
| 95  | 202188_at   | NM_014669 | KIAA0095 | KIAA0095 gene product   |
| 96  | 202219_at   | NM_005629 | SLC6A8   | solute carrier family 6, member 8   |
| 97  | 202224_at   | NM_016823 |          | v-crk avian sarcoma virus CT10 oncogene homolog   |
| 98  | 202225_at   | NM_016823 |          | v-crk avian sarcoma virus CT10 oncogene homolog   |
| 99  | 202260_s_at | NM_003165 | STXBP1   | syntaxin binding protein 1  |
| 100 | 202295_s_at | NM_004390 | CTSH     | cathepsin H   |
| 101 | 202329_at   | NM_004383 | CSK      | c-src tyrosine kinase   |
| 102 | 202367_at   | NM_001913 | CUTL1    | cut (Drosophila)-like 1   |
| 103 | 202370_s_at | NM_001755 | CBFB     | core-binding factor, $\beta$ sub trans var 2  |
| 104 | 202478_at   | NM_021643 | GS3955   | GS3955 protein  |
| 105 | 202503_s_at | NM_014736 | KIAA0101 | KIAA0101 gene product   |
| 106 | 202589_at   | NM_001071 | TYMS     | thymidylate synthetase  |
| 107 | 202603_at   | N51370    |          | disintegrin and metalloproteinase domain 10   |
| 108 | 202705_at   | NM_004701 | CCNB2    | cyclin B2   |
| 109 | 202737_s_at | NM_012321 | LSM4     | U6 snRNA-associated Sm-like protein   |
| 110 | 202779_s_at | NM_014501 | E2-EPF   | ubiquitin carrier protein   |
| 111 | 202785_at   | NM_005001 | NDUFA7   | NADH dehydrogenase 1 $\alpha$ subcomplex, 7   |
| 112 | 202862_at   | NM_000137 | FAH      | fumarylacetoacetate   |
| 113 | 202898_at   | NM_014654 | KIAA0468 | KIAA0468 gene product   |
| 114 | 202954_at   | NM_007019 | UBCH10   | ubiquitin carrier protein E2-C  |
| 115 | 202958_at   | NM_002833 | PTPN9    | protein tyrosine phosphatase, non-receptor type 9   |
| 116 | 202961_s_at | NM_004889 | ATP5J2   | ATP synthase, H <sup>+</sup> transporting, mitochondrial F0 complex, subunit f, isoform 2 |
| 117 | 202986_at   | NM_014862 | KIAA0307 | KIAA0307 gene product   |
| 118 | 203011_at   | NM_005536 | IMPA1    | inositol(myo)-1(or 4)-monophosphatase 1   |
| 119 | 203022_at   | NM_006397 | RNASEHI  | ribonuclease HI, large subunit  |
| 120 | 203069_at   | NM_014849 | KIAA0736 | KIAA0736 gene product   |
| 121 | 203071_at   | NM_004636 | SEMA3B   | sema domain, Ig domain, short basic domain, secreted, 3B                                  |
| 122 | 203094_at   | NM_014628 | KIAA0110 | gene predicted from cDNA  |
| 123 | 203145_at   | NM_006461 | DEEPEST  | mitotic spindle coiled-coil related   |
| 124 | 203167_at   | NM_003255 | TIMP2    | tissue inhibitor of metalloproteinase 2   |
| 125 | 203217_s_at | NM_003896 | SIAT9    | sialyltransferase 9   |
| 126 | 203234_at   | NM_003364 | UP       | uridine phosphorylase   |
| 127 | 203256_at   | NM_001793 | CDH3     | cadherin 3, type 1, P-cadherin (placental)  |
| 128 | 203262_s_at | NM_004699 | DXS9928E | chromosome X 9928 expressed seq   |
| 129 | 203300_x_at | NM_003916 | AP1S2    | adaptor-related protein complex 1, sigma 2 subunit  |
| 130 | 203315_at   | BC000103  |          | NCK adaptor protein 2,  |
| 131 | 203366_at   | NM_002693 | POLG     | polymerase (DNA directed), gamma  |
| 132 | 203396_at   | NM_002789 | PSMA4    | proteasome subunit, $\alpha$ type, 4  |
| 133 | 203452_at   | NM_012200 | B3GAT3   | beta-1,3-glucuronyltransferase 3  |
| 134 | 203456_at   | NM_007213 | JM4      | JM4 protein   |
| 135 | 203502_at   | NM_001724 | BPGM     | 2,3-bisphosphoglycerate mutase  |
| 136 | 203518_at   | NM_000081 | CHS1     | Chediak-Higashi syndrome 1  |
| 137 | 203554_x_at | NM_004219 | PTTG1    | pituitary tumor-transforming 1  |

|     |             |           |          |  |
|-----|-------------|-----------|----------|--|
| 138 | 203557_s_at | NM_000281 | PCBD     | 6-pyruvoyl-tetrahydropterin synthasedimerization cofactor of hepatocyte nuclear factor 1 alpha   |
| 139 | 203570_at   | NM_005576 | LOXL1    | lysyl oxidase-like 1   |
| 140 | 203590_a t  | NM_006141 | DNCL12   | dynein, cytoplasmic, light intermediate polypeptide 2  |
| 141 | 203643_at   | NM_006494 | ERF      | Ets2 repressor factor  |
| 142 | 203663_s_at | NM_004255 | COX5A    | cytochrome c oxidase subunit Va  |
| 143 | 203668_at   | NM_006715 | MAN2C1   | mannosidase, $\alpha$ , class 2C, mem 1  |
| 144 | 203693_s_at | NM_001949 | E2F3     | E2F transcription factor 3   |
| 145 | 203695_s_at | NM_004403 | DFNA5    | deafness, autosomal dominant 5   |
| 146 | 203723_at   | NM_002221 | ITPKB    | inositol 1,4,5-trisphosphate 3-kinase B  |
| 147 | 203729_at   | NM_001425 | EMP3     | epithelial membrane protein 3  |
| 148 | 203730_s_at | BF196931  | ZFP95    | zinc finger protein homologous to Zfp95 in mouse   |
| 149 | 203731_s_at | NM_014569 | ZFP95    |  |
| 150 | 203775_at   | NM_014251 | SLC25A13 | solute carrier family 25, member 13  |
| 151 | 203827_at   | NM_017983 | FLJ10055 | hypothetical protein FLJ10055  |
| 152 | 203878_s_at | NM_005940 | MMP11    | matrix metalloproteinase 11  |
| 153 | 204014_at   | NM_001394 | DUSP4    | dual specificity phosphatase 4   |
| 154 | 204015_s_at | BC002671  | DUSP4    |  |
| 155 | 204033_at   | NM_004237 | TRIP13   | thyroid hormone receptor interactor 13   |
| 156 | 204092_s_at | NM_003600 | STK15    | serinethreonine kinase 15  |
| 157 | 204099_at   | NM_003078 | SMARCD3  | SWISNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 3 |
| 158 | 204170_s_at | NM_001827 | CKS2     | CDC28 protein kinase 2   |
| 159 | 204197_s_at | NM_004350 | RUNX3    | runt-related transcription factor 3  |
| 160 | 204198_s_at | AA541630  | RUNX3    |  |
| 161 | 204202_at   | NM_017604 | KIAA1023 | KIAA1023 protein   |
| 162 | 204228_at   | NM_006347 | USA-CYP  | cyclophilin  |
| 163 | 204244_s_at | NM_006716 | ASK      | activator of S phase kinase  |
| 164 | 204247_s_at | NM_004935 | CDK5     | cyclin-dependent kinase 5  |
| 165 | 204252_at   | M68520    |          | cdc2-related protein kinase  |
| 166 | 204262_s_at | NM_000447 | PSEN2    | presenilin 2 transcript variant 1  |
| 167 | 204423_at   | NM_013255 | MKLN1    | muskelin 1, intracellular mediator containing kelch motifs                                       |
| 168 | 204436_at   | NM_025201 | PP1628   | hypothetical protein PP1628  |
| 169 | 204458_at   | AL110209  |          | DKFZp564A0122  |
| 170 | 204467_s_at | NM_000345 | SNCA     | synuclein, $\alpha$ transcript var NACP140   |
| 171 | 204584_at   | AI653981  | L1CAM    | L1 cell adhesion molecule, MASA transcript var 1   |
| 172 | 204585_s_at | NM_000425 | L1CAM    |  |
| 173 | 204647_at   | NM_004838 | HOMER-3  | Homer, neuronal imm early gene, 3  |
| 174 | 204654_s_at | NM_003220 | TFAP2A   | transcription factor AP-2 alpha  |
| 175 | 204709_s_at | NM_004856 | KNSL5    | kinesin-like 5   |
| 176 | 204778_x_at | AW102783  | HOXB7    | homeo box B7   |
| 177 | 204779_s_at | NM_004502 | HOXB7    |  |
| 178 | 204857_at   | NM_003550 | MAD1L1   | MAD1-like 1  |
| 179 | 204932_at   | BF433902  |          | TNF receptor superfam, mem 11b   |
| 180 | 204973_at   | NM_000166 | GJB1     | gap junction protein, beta 1, 32kD   |
| 181 | 204995_at   | AL567411  |          | cyclin-dependent kinase 5, regulatory sub 1 (p35)  |
| 182 | 205051_s_at | NM_000222 | KIT      | v-kit Hardy-Zuckerman 4 feline   |

|     |             |           |          |   |
|-----|-------------|-----------|----------|---|
|     |             |           |          | sarcoma viral oncogene homolog  |
| 183 | 205142_x_at | NM_000033 | ABCD1    | ATP-binding cassette, sub-family D (ALD), mem 1   |
| 184 | 205169_at   | NM_005057 | RBBP5    | retinoblastoma-binding protein 5  |
| 185 | 205373_at   | NM_004389 | CTNNA2   | catenin alpha 2   |
| 186 | 205376_at   | NM_003866 | INPP4B   | inositol polyphosphate-4-phosphatase, type II, 105kD  |
| 187 | 205405_at   | NM_003966 | SEMA5A   | sema domain, seven thrombospondin repeats, transmembrane domain and short cytoplasmic domain 5A |
| 188 | 205447_s_at | BE222201  |          | mitogen-activated protein kinase kinase kinase 12   |
| 189 | 205458_at   | BG034972  |          | melanocortin 1 receptor   |
| 190 | 205566_at   | NM_007011 | HS1-2    | putative transmembrane protein  |
| 191 | 205591_at   | NM_006334 | AMY      | neuroblastoma (nerve tissue) protein  |
| 192 | 205681_at   | NM_004049 | BCL2A1   | BCL2-related protein A1   |
| 193 | 205690_s_at | NM_003910 | G10      | maternal G10 transcript   |
| 194 | 205691_at   | NM_004209 | SYNGR3   | synaptogyrin 3  |
| 195 | 205717_x_at | NM_002588 | PCDHGC3  | protocadherin gamma subfamily C, 3  |
| 196 | 205813_s_at | NM_000429 | MAT1A    | methionine adenosyltransferase I, $\alpha$  |
| 197 | 205937_at   | NM_006569 | CGR11    | cell growth regulatory with EF-hand domain  |
| 198 | 205945_at   | NM_000565 | IL6R     | interleukin 6 receptor  |
| 199 | 205996_s_at | NM_013411 | AK2 B    | adenylate kinase 2  |
| 200 | 206128_at   | AI264306  |          | adrenergic, alpha-2C-, receptor   |
| 201 | 206307_s_at | NM_004472 | FOXD1    | forkhead box D1   |
| 202 | 206332_s_at | NM_005531 | IFI16    | interferon, gamma-inducible 16  |
| 203 | 206397_x_at | NM_001492 | GDF1     | growth differentiation factor 1   |
| 204 | 206441_s_at | NM_017828 | FLJ20452 | hypothetical protein FLJ20452   |
| 205 | 206462_s_at | NM_002530 | NTRK3    | neurotrophic tyrosine kinase, receptor, type 3  |
| 206 | 206503_x_at | NM_002675 | PML      | promyelocytic leukemia  |
| 207 | 206617_s_at | NM_002910 | RENBP    | renin-binding protein   |
| 208 | 206630_at   | NM_000372 | TYR      | tyrosinase  |
| 209 | 206688_s_at | NM_006693 | CPSF4    | cleavage and polyadenylation specific factor 4, 30kD subunit                                    |
| 210 | 206696_at   | NM_000273 | OA1      | ocular albinism 1   |
| 211 | 206777_s_at | NM_000496 | CRYBB2   | crystallin, beta B2   |
| 212 | 206864_s_at | NM_003806 | HRK      | harakiri, BCL2-interacting protein  |
| 213 | 206976_s_at | NM_006644 | HSP105B  | heat shock 105kD  |
| 214 | 207038_at   | NM_004694 | SLC16A6  | solute carrier family 16 member 6   |
| 215 | 207060_at   | NM_001427 | EN2      | engrailed homolog 2   |
| 216 | 207144_s_at | NM_004143 | CITED1   | Cbpb300-interacting transactivator, with GluAsp-rich carboxy-terminal domain, 1                 |
| 217 | 207163_s_at | NM_005163 | AKT1     | v-akt murine thymoma viral oncogene homolog 1   |
| 218 | 207183_at   | NM_006143 | GPR19    | G protein-coupled receptor 19   |
| 219 | 207592_s_at | NM_001194 | HCN2     | hyperpolarization activated cyclic nucleotide-gated potassium channel 2                         |
| 220 | 207614_s_at | NM_003592 | CUL1     | cullin 1  |
| 221 | 207622_s_at | NM_005692 | ABCF2    | ATP-binding cassette, sub-fam F mem 2   |
| 222 | 207828_s_at | NM_005196 | CENPF    | centromere protein F  |

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|-----|-------------|-----------|---------------|---|
| 223 | 208002_s_at | NM_007274 | HBACH         | cytosolic acyl coenzyme A thioester hydrolase             |
| 224 | 208089_s_at | NM_030794 | FLJ21007      | hypothetical protein FLJ21007                             |
| 225 | 208308_s_at | NM_000175 | GPI           | glucose phosphate isomerase                               |
| 226 | 208540_x_at | NM_021039 | S100A14       | S100 calcium-binding protein A14                          |
| 227 | 208644_at   | M32721    |               | poly(ADP-ribose) polymerase                               |
| 228 | 208657_s_at | AF142408  |               | cell division control protein septin D1                   |
| 229 | 208677_s_at | AL550657  |               | Basigin   |
| 230 | 208696_at   | AF275798  |               | PNAS-102  |
| 231 | 208710_s_at | AI424923  |               | adaptor-related protein complex 3, delta 1 subunit        |
| 232 | 208723_at   | BC000350  |               | ubiquitin specific protease 11                            |
| 233 | 208744_x_at | BG403660  |               | heat shock 105kD  |
| 234 | 208837_at   | BC000027  |               | integral type I protein                                   |
| 235 | 208916_at   | AF105230  | SLC1A5        | neutral amino acid transporter                            |
| 236 | 208928_at   | AF258341  |               | NADPH-cytochrome P450 reductase                           |
| 237 | 208956_x_at | U62891    | DUT           | deoxyuridine triphosphatase                               |
| 238 | 208974_x_at | BC003572  |               | karyopherin (importin) beta 1                             |
| 239 | 208975_s_at | L38951    |               | importin beta subunit                                     |
| 240 | 209015_s_at | BC002446  |               | MRJ gene for a member of DNAJ fam                         |
| 241 | 209036_s_at | BC001917  |               | malate dehydrogenase 2, NAD                               |
| 242 | 209053_s_at | AF083389  |               | Wolf-Hirschhorn syn candidate 1                           |
| 243 | 209072_at   | M13577    | MBP           | myelin basic protein                                      |
| 244 | 209079_x_at | AF152318  | PCDH-gamma-A1 | protocadherin gamma A1                                    |
| 245 | 209081_s_at | NM_030582 | COL18A1       | collagen, type XVIII, alpha 1                             |
| 246 | 209123_at   | BC000576  |               | quinoid dihydropteridine reductase                        |
| 247 | 209132_s_at | BE313890  |               | hypothetical protein FLJ20452                             |
| 248 | 209172_s_at | U30872    |               | mitosin   |
| 249 | 209197_at   | AA626780  |               | KIAA0080 protein  |
| 250 | 209198_s_at | BC004291  |               | Similar to synaptotagmin 11                               |
| 251 | 209247_s_at | BC001661  |               | ATP-binding cassette, sub-fam F mem 2                     |
| 252 | 209254_at   | AF277177  |               | KIAA0265 protein  |
| 253 | 209255_at   | AF277177  |               | KIAA0265 protein  |
| 254 | 209256_s_at | AF277177  |               | PNAS-119  |
| 255 | 209283_at   | AF007162  |               | unknown mRNA  |
| 256 | 209345_s_at | AL561930  |               | phosphatidylinositol 4-kinase type II                     |
| 257 | 209407_s_at | AF068892  |               | Dukes type B colon adenocarcinoma truncated suppressin    |
| 258 | 209515_s_at | U38654    |               | Rab27a  |
| 259 | 209773_s_at | BC001886  |               | ribonucleotide reductase M2 polypep                       |
| 260 | 209825_s_at | BC002906  |               | Sim to uridine monophosphate kinase                       |
| 261 | 209827_s_at | NM_004513 | IL16          | interleukin 16  |
| 262 | 209828_s_at | M90391    |               | putative IL-16 protein precursor                          |
| 263 | 209848_s_at | U01874    |               | me20m   |
| 264 | 209875_s_at | M83248    |               | nephropontin  |
| 265 | 209932_s_at | U90223    |               | deoxyuridine triphosphate nucleotidohydrolase precursor   |
| 266 | 210052_s_at | AF098158  |               | restricted expressed proliferation associated protein 100 |
| 267 | 210073_at   | L32867    |               | alpha 2,8-sialyltransferase                               |
| 268 | 210111_s_at | AF277175  |               | PNAS-138  |
| 269 | 210127_at   | BC002510  |               | small GTPase RAB6B  |

|     |             |          |               |   |
|-----|-------------|----------|---------------|---|
| 270 | 210854_x_at | U17986   |               | GABAnoradrenaline transporter                         |
| 271 | 210926_at   | AY014272 | FKSG30        | FKSG30  |
| 272 | 210948_s_at | AF294627 | LEF1          | lymphoid enhancer factor 1 isoform                    |
| 273 | 210951_x_at | AF125393 |               | Rab27 isoform   |
| 274 | 211013_x_at | AF230411 |               | tripartite motif protein TRIM19 lambda                |
| 275 | 211052_s_at | BC006364 |               | clone MGC:12705,                                      |
| 276 | 211066_x_at | BC006439 |               | Similar to protocadherin gamma subfamily A, 5         |
| 277 | 211373_s_at | U34349   | AD3LPAD5      | seven trans-membrane domain                           |
| 278 | 211564_s_at | BC003096 |               | Sim to LIM domain protein                             |
| 279 | 211752_s_at | BC005954 |               | clone MGC:14592                                       |
| 280 | 211759_x_at | BC005969 |               | clone MGC:14625                                       |
| 281 | 211833_s_at | U19599   |               | BAX delta   |
| 282 | 212000_at   | AB002363 |               | KIAA0365 gene product                                 |
| 283 | 212070_at   | AL554008 |               | G protein-coupled receptor 56                         |
| 284 | 212081_x_at | AF129756 | MSH55         | MSH55   |
| 285 | 212119_at   | BF348067 |               | phosphatidylinositol glycan, class F                  |
| 286 | 212178_s_at | AK022555 |               | FLJ12493 fis  |
| 287 | 212193_s_at | BE881529 |               | KIAA0731 protein                                      |
| 288 | 212247_at   | AW008531 |               | KIAA0225 protein                                      |
| 289 | 212285_s_at | AF016903 |               | IMAGE:3506210   |
| 290 | 212312_at   | AL117381 |               | clone RP5-857M17 on chrom 20                          |
| 291 | 212338_at   | AA621962 |               | KIAA0727 protein                                      |
| 292 | 212402_at   | BE895685 |               | KIAA0853 protein                                      |
| 293 | 212472_at   | BE965029 |               | FLJ22463 fis  |
| 294 | 212473_s_at | BE965029 |               | FLJ22463 fis  |
| 295 | 212512_s_at | AA551784 |               | coactivator-associated arginine methyltransferase-1   |
| 296 | 212520_s_at | AI684141 |               | SWISNF related  |
| 297 | 212552_at   | BE617588 |               | hippocalcin-like                                      |
| 298 | 212715_s_at | AB020626 |               | KIAA0819 protein                                      |
| 299 | 212739_s_at | AL523860 |               | non-metastatic cells 4                                |
| 300 | 212744_at   | AI813772 |               | clone HQ0692  |
| 301 | 212745_s_at | AI813772 |               | clone HQ0692  |
| 302 | 212793_at   | BF513244 |               | KIAA0381 protein                                      |
| 303 | 212796_s_at | BF195608 |               | KIAA1055 protein                                      |
| 304 | 213002_at   | BF347326 | MARCKS, 80K-L | myristoylated alanine-rich protein kinase C substrate |
| 305 | 213007_at   | BG478677 |               | polymerase (DNA directed), gamma                      |
| 306 | 213008_at   | BG478677 |               | polymerase (DNA directed), gamma                      |
| 307 | 213096_at   | T51252   |               | KIAA0481 gene product                                 |
| 308 | 213131_at   | R38389   |               | olfactomedin related ER localized                     |
| 309 | 213169_at   | BG109855 |               | clone TUA8 Cri-du-chat region                         |
| 310 | 213215_at   | AI910895 |               | clone EUROIMAGE 42138                                 |
| 311 | 213217_at   | AU149572 |               | adenylate cyclase 2(brain)                            |
| 312 | 213241_at   | AF035307 |               | clone 23785   |
| 313 | 213274_s_at | BE875786 |               | cathepsin B   |
| 314 | 213275_x_at | BE875786 |               | cathepsin B   |
| 315 | 213330_s_at | BE886580 |               | stress-induced-phosphoprotein 1                       |
| 316 | 213333_at   | AL520774 |               | malate dehydrogenase 2, NAD                           |
| 317 | 213338_at   | BF062629 |               | DKFZP586E1621 protein                                 |
| 318 | 213392_at   | AW070229 |               | G protein-coupled receptor, fam C, group 5, mem B     |
| 319 | 213474_at   | AI890903 |               | ESTs  |

|     |             |           |                   |   |
|-----|-------------|-----------|-------------------|---|
| 320 | 213496_at   | AW592563  |                   | KIAA0455 gene product   |
| 321 | 213573_at   | AA861608  |                   | karyopherin (importin) beta 1   |
| 322 | 213587_s_at | AI884867  |                   | ribosomal protein L26   |
| 323 | 213638_at   | AW054711  |                   | PAC 257A7 on chromosome 6p24  |
| 324 | 213670_x_at | AI768378  |                   | KIAA0618 gene product   |
| 325 | 213720_s_at | AI831675  |                   | SWISNF related, matrix associated, actin dependent regulator of chromatin, subfam a, member 4 |
| 326 | 213746_s_at | AW051856  |                   | filamin A, alpha  |
| 327 | 213836_s_at | AW052084  |                   | KIAA1001 protein  |
| 328 | 213895_at   | BF445047  |                   | epithelial membrane protein 1   |
| 329 | 213960_at   | T87225    |                   | CLONE=IMAGE:22392   |
| 330 | 214023_x_at | AL533838  |                   | tubulin, beta polypeptide   |
| 331 | 214068_at   | AF070610  |                   | clone 24505   |
| 332 | 214104_at   | AI703188  |                   | G-protein coupled receptor  |
| 333 | 214201_x_at | AA742237  |                   | HLA-B associated transcript-2   |
| 334 | 214581_x_at | BE568134  |                   | death receptor 6  |
| 335 | 214614_at   | AI738662  |                   | homeo box HB9   |
| 336 | 214632_at   | AA295257  |                   | neuropilin 2  |
| 337 | 214656_x_at | BE790157  |                   | myosin IB   |
| 338 | 214687_x_at | AK026577  |                   | FLJ22924 fis  |
| 339 | 214708_at   | BG484314  |                   | syntrophin, beta 1  |
| 340 | 214710_s_at | BE407516  |                   | cyclin B1   |
| 341 | 214714_at   | AK022360  |                   | FLJ12298 fis  |
| 342 | 214717_at   | AL137534  |                   | DKFZp434H1419   |
| 343 | 214752_x_at | AI625550  |                   | filamin A, alpha  |
| 344 | 214778_at   | AB011541  |                   | MEGF8   |
| 345 | 214841_at   | AF070524  |                   | clone 24453   |
| 346 | 214893_x_at | AI421964  |                   | hyperpolarization activated cyclic nucleotide-gated potassium channel 2                       |
| 347 | 214896_at   | AL109671  |                   | EUROIMAGE 29222   |
| 348 | 215025_at   | S76476    |                   | trkC {alternatively spliced}  |
| 349 | 215115_x_at | AI613045  |                   | ets variant gene 6 (TEL oncogene)   |
| 350 | 215126_at   | AL109716  |                   | EUROIMAGE 208948  |
| 351 | 215155_at   | J04178    | HEXA              | abnormal $\beta$ -hexosaminidase $\alpha$ chain   |
| 352 | 215311_at   | AL109696  |                   | EUROIMAGE 21920   |
| 353 | 215812_s_at | U41163    | SLC6A10           | creatine transporter  |
| 354 | 215836_s_at | AK026188  |                   | FLJ22535 fis  |
| 355 | 216194_s_at | AD001527  |                   | DNA from chrom 19-cosmid f24590 containing CAPNS and POL2RI                                   |
| 356 | 216973_s_at | S49765    |                   | homeo box B7  |
| 357 | 217033_x_at | S76475    | trkC              | neurotrophic tyrosine kinase, receptor, type 3  |
| 358 | 217104_at   | AL109714  |                   | EUROIMAGE 327506  |
| 359 | 217226_s_at | M95929    | PHOX1             | Paired mesoderm homeo box 1   |
| 360 | 217297_s_at | AF143684  | MYO9b             | unconventional myosin IXb   |
| 361 | 217377_x_at | AF041811  | ETV6-NTRK3 fusion | ETS related protein-growth factor receptor tyrosine kinase fusion proteins                    |
| 362 | 217419_x_at | AK021586  |                   | FLJ11524 fis  |
| 363 | 217624_at   | AA464753  |                   | ESTs  |
| 364 | 217799_x_at | NM_003344 | UBE2H             | ubiquitin-conjugating enzyme E2H  |
| 365 | 217827_s_at | NM_016630 | ACP33             | acid cluster protein 33   |
| 366 | 217867_x_at | NM_012105 | BACE2             | beta-site APP-cleaving enzyme 2   |

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|-----|-------------|-----------|-----------|--|
| 367 | 217874_at   | NM_003849 | SUCLG1    | succinate-CoA ligase, GDP-forming, alpha subunit         |
| 368 | 217891_at   | NM_022744 | FLJ13868  | hypothetical protein FLJ13868                            |
| 369 | 218009_s_at | NM_003981 | PRC1      | protein regulator of cytokinesis 1                       |
| 370 | 218030_at   | NM_014030 | GIT1      | G protein-coupled receptor kinase-interactor 1           |
| 371 | 218074_at   | NM_016062 | LOC51647  | CGI-128 protein  |
| 372 | 218143_s_at | NM_005697 | SCAMP2    | secretory carrier membrane protein 2                     |
| 373 | 218151_x_at | NM_024531 | FLJ11856  | hypothetical protein FLJ11856                            |
| 374 | 218152_at   | NM_018200 | HMG20A    | high-mobility group 20A                                  |
| 375 | 218161_s_at | NM_017882 | FLJ20561  | hypothetical protein FLJ20561                            |
| 376 | 218175_at   | NM_025140 | FLJ22471  | hypothetical protein FLJ22471                            |
| 377 | 218330_s_at | NM_018162 | FLJ10633  | hypothetical protein FLJ10633                            |
| 378 | 218349_s_at | NM_017975 | FLJ10036  | hypothetical protein FLJ10036                            |
| 379 | 218359_at   | NM_024958 | FLJ23329  | hypothetical protein FLJ23329                            |
| 380 | 218376_s_at | NM_022765 | FLJ11937  | hypothetical protein FLJ11937                            |
| 381 | 218447_at   | NM_020188 | DC13      | DC13 protein   |
| 382 | 218542_at   | NM_018131 | FLJ10540  | hypothetical protein FLJ10540                            |
| 383 | 218564_at   | BC002574  | FLJ10520  | hypothetical protein FLJ10520                            |
| 384 | 218618_s_at | NM_022763 | FLJ23399  | hypothetical protein FLJ23399                            |
| 385 | 218678_at   | NM_024609 | FLJ21841  | hypothetical protein FLJ21841                            |
| 386 | 218774_at   | NM_014026 | HSPC015   | HSPC015 protein  |
| 387 | 218786_at   | NM_016575 | TU12B1-TY | TU12B1-TY protein  |
| 388 | 218824_at   | NM_018215 | FLJ10781  | hypothetical protein FLJ10781                            |
| 389 | 218839_at   | NM_012258 | HEY1      | hairyhancer-of-split related with YRPW motif 1           |
| 390 | 218856_at   | NM_016629 | LOC51323  | hypothetical protein LOC51323                            |
| 391 | 218888_s_at | NM_018092 | FLJ10430  | hypothetical protein FLJ10430                            |
| 392 | 218931_at   | NM_022449 | FLJ12538  | hypothetical protein FLJ12538                            |
| 393 | 218952_at   | NM_013271 | SAAS      | granin-like neuroendocrine peptide precursor             |
| 394 | 218956_s_at | NM_015545 | KIAA0632  | KIAA0632 protein   |
| 395 | 218980_at   | NM_025135 | KIAA1695  | hypothetical protein FLJ22297                            |
| 396 | 218996_at   | NM_013342 | TFPT      | TCF3 (E2A) fusion partner                                |
| 397 | 219011_at   | NM_020904 | PLEKHA4   | pleckstrin homology domain-containing, family A member 4 |
| 398 | 219039_at   | NM_017789 | FLJ20369  | hypothetical protein FLJ20369                            |
| 399 | 219040_at   | NM_024535 | FLJ22021  | hypothetical protein FLJ22021                            |
| 400 | 219041_s_at | NM_014374 | AP4       | zinc finger protein                                      |
| 401 | 219051_x_at | NM_024042 | MGC2601   | hypothetical protein MGC2601                             |
| 402 | 219066_at   | NM_021823 | MDS018    | hypothetical protein MDS018                              |
| 403 | 219066_at   | NM_021823 | MDS018    | hypothetical protein MDS018                              |
| 404 | 219148_at   | NM_018492 | TOPK      | PDZ-binding kinase; T-cell originated protein kinase     |
| 405 | 219152_at   | NM_015720 | PODLX2    | endoglycan   |
| 406 | 219219_at   | NM_017854 | FLJ20512  | hypothetical protein FLJ20512                            |
| 407 | 219361_s_at | NM_022767 | FLJ12484  | hypothetical protein FLJ12484                            |
| 408 | 219372_at   | NM_014055 | CDV-1     | CDV-1 protein  |
| 409 | 219408_at   | NM_019023 | FLJ10640  | hypothetical protein FLJ10640                            |
| 410 | 219478_at   | NM_021197 | WFDC1     | WAP four-disulfide core domain 1                         |
| 411 | 219491_at   | NM_024036 | MGC3103   | hypothetical protein MGC3103                             |
| 412 | 219522_at   | NM_014344 | FJX1      | putative secreted ligand homologous to fjx1              |
| 413 | 219537_x_at | NM_016941 | DLL3      | Delta (Drosophila)-like 3                                |

|     |             |           |           |  |
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| 414 | 219555_s_at | NM_018455 | BM039     | uncharacterized bone marrow protein BM039  |
| 415 | 219578_s_at | NM_030594 | FLJ13203  | hypothetical protein FLJ13203  |
| 416 | 219634_at   | NM_018413 | C4ST      | chondroitin 4-sulfotransferase   |
| 417 | 219637_at   | NM_025139 | FLJ12584  | hypothetical protein FLJ12584  |
| 418 | 219703_at   | NM_018365 | FLJ11222  | hypothetical protein FLJ11222  |
| 419 | 219742_at   | NM_030567 | MGC10772  | hypothetical protein MGC10772  |
| 420 | 219895_at   | NM_017938 | FLJ20716  | hypothetical protein FLJ20716  |
| 421 | 219933_at   | NM_016066 | LOC51022  | CGI-133 protein  |
| 422 | 220116_at   | NM_021614 | KCNN2     | potassium intermediatesmall conductance calcium-activated channel, subfamily N, member 2 |
| 423 | 220155_s_at | NM_023924 | FLJ13441  | hypothetical protein FLJ13441  |
| 424 | 220178_at   | NM_021731 | PP3501    | hypothetical protein PP3501  |
| 425 | 220454_s_at | NM_020796 | SEMA6A    | sema domain, transmembrane domain and cytoplasmic domain, 6A                             |
| 426 | 220864_s_at | NM_015965 | LOC51079  | CGI-39 protein; cell death-regulatory protein GRIM19                                     |
| 427 | 220948_s_at | NM_000701 | ATP1A1    | ATPase, Na+K+ transporting, alpha 1 polypeptide  |
| 428 | 220973_s_at | NM_030974 |           | hypothetical protein DKFZp434N1923   |
| 429 | 220974_x_at | NM_030971 | BA108L7.2 | similar to rat tricarboxylate carrier-like   |
| 430 | 220980_s_at | NM_031284 |           | hypothetical protein DKFZp434B195  |
| 431 | 221059_s_at | NM_021615 | CHST6     | carbohydrate sulfotransferase 6  |
| 432 | 221483_s_at | AF084555  | ARPP-19   | okadaic acid-inducible and cAMP-regulated phosphoprotein 19                              |
| 433 | 221484_at   | NM_004776 |           | UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 5                         |
| 434 | 221538_s_at | AL136663  |           | DKFZp564A176   |
| 435 | 221558_s_at | AF288571  | LEF1      | lymphoid enhancer factor-1   |
| 436 | 221577_x_at | AF003934  |           | prostate differentiation factor  |
| 437 | 221641_s_at | AF241787  |           | CGI16-iso  |
| 438 | 221688_s_at | AL136913  |           | DKFZp586L0118  |
| 439 | 221710_x_at | BC006241  |           | hypothetical protein FLJ10647  |
| 440 | 221732_at   | AK026161  |           | FLJ22508 fis   |
| 441 | 221759_at   | AL583123  |           | CLONE=CS0DL009YN09   |
| 442 | 221797_at   | AY007126  |           | clone CDABP0028  |
| 443 | 221799_at   | AB037823  |           | KIAA1402 protein,  |
| 444 | 221815_at   | BE671816  |           | ESTs   |
| 445 | 221882_s_at | AI636233  |           | five-span transmembrane protein M83  |
| 446 | 221902_at   | AL567940  |           | CLONE=CS0DF036YK19   |
| 447 | 221909_at   | BF984207  |           | ESTs   |
| 448 | 221962_s_at | AI829920  |           | ubiquitin-conjugating enzyme E2H   |
| 449 | 222116_s_at | AL157485  |           | DKFZp762O207   |
| 450 | 222153_at   | AK023133  |           | FLJ13071 fis   |
| 451 | 222155_s_at | AK021918  |           | FLJ11856 fis   |
| 452 | 222175_s_at | AK000003  |           | FLJ00003 protein   |
| 453 | 222196_at   | AK000470  |           | FLJ20463 fis   |
| 454 | 222199_s_at | AK001289  |           | FLJ10427 fis   |
| 455 | 222206_s_at | AA781143  |           | EUROIMAGE 2021883  |
| 456 | 222212_s_at | AK001105  |           | FLJ10243 fis   |
| 457 | 222231_s_at | AK025328  |           | FLJ21675 fis   |
| 458 | 222234_s_at | AK022644  |           | FLJ12582 fis   |
| 459 | 222240_s_at | AL137749  |           | DKFZp434A0612  |



|     |             |           |            |   |
|-----|-------------|-----------|------------|---|
| 460 | 222294_s_at | AW971415  |            | ESTs  |
| 461 | 32811_at    | X98507    |            | myosin-I beta   |
| 462 | 40560_at    | U28049    | TXB2       | TBX2  |
| 463 | 44783_s_at  | R61374    |            | IMAGE-37665   |
| 464 | 46665_at    | AI949392  |            | IMAGE-2470926   |
| 465 | 55093_at    | AA534198  |            | IMAGE-993116  |
| 466 | 63825_at    | AI557319  |            |   |
| 467 | 87100_at    | AI832249  |            |   |
| 468 | 200017_at   | NM_002954 | RPS27A     | ribosomal protein S27a  |
| 469 | 200606_at   | NM_004415 | DSP        | desmoplakin (DPI, DPII)   |
| 470 | 200632_s_at | NM_006096 | NDRG1      | N-myc downstream regulated  |
| 471 | 200636_s_at | NM_002840 | PTPRF      | protein tyrosine phosphatase, receptor type, F                                  |
| 472 | 200795_at   | NM_004684 | SPARCL1    | SPARC-like 1  |
| 473 | 200810_s_at | NM_001280 | CIRBP      | cold inducible RNA-binding protein  |
| 474 | 200897_s_at | NM_016081 | KIAA0992   | Pailadin  |
| 475 | 200953_s_at | NM_001759 | CCND2      | cyclin D2   |
| 476 | 200965_s_at | NM_006720 | ABLIM-s    | actin binding LIM protein 1 transcript variant                                  |
| 477 | 201012_at   | NM_000700 | ANXA1      | annexin A1  |
| 478 | 201041_s_at | NM_004417 | DUSP1      | dual specificity phosphatase 1  |
| 479 | 201125_s_at | NM_002213 | ITGB5      | integrin, beta 5  |
| 480 | 201200_at   | NM_003851 | CREG       | cellular repressor of E1A-stimulated genes                                      |
| 481 | 201286_at   | Z48199    | syndecan 1 | syndecan-1 gene (exons 2-5)   |
| 482 | 201328_at   | AL575509  |            | v-ets avian erythroblastosis virus E26 oncogene homolog 2                       |
| 483 | 201425_at   | NM_000690 | ALDH2      | aldehyde dehydrogenase 2, mitochondrial   |
| 484 | 201427_s_at | NM_005410 | SEPP1      | selenoprotein P, plasma, 1  |
| 485 | 201432_at   | NM_001752 | CAT        | Catalase  |
| 486 | 201540_at   | NM_001449 | FHL1       | four and a half LIM domains 1   |
| 487 | 201667_at   | NM_000165 | GJA1       | gap junction protein, alpha 1, 43kD   |
| 488 | 201681_s_at | AB011155  | KIAA0583   | KIAA0583  |
| 489 | 201798_s_at | NM_013451 | FER1L3     | fer-1 (C.elegans)-like 3 (myoferlin)  |
| 490 | 201820_at   | NM_000424 | KRT5       | keratin 5   |
| 491 | 201829_at   | AW263232  | NET1       | neuroepithelial cell transf gene 1  |
| 492 | 201830_s_at | NM_005863 | NET1       | neuroepithelial cell transf gene 1  |
| 493 | 201839_s_at | NM_002354 | TACSTD1    | tumor-associated calcium signal transducer 1                                    |
| 494 | 201842_s_at | AI826799  |            | EGF-containing fibulin-like extracellular matrix protein 1                      |
| 495 | 201843_s_at | NM_004105 | EFEMP1     | EGF-containing fibulin-like extracellular matrix protein 1 transcript variant 1 |
| 496 | 201983_s_at | AW157070  |            | epidermal growth factor receptor  |
| 497 | 201984_s_at | NM_005228 | EGFR       | epidermal growth factor receptor  |
| 498 | 202054_s_at | NM_000382 | ALDH3A2    | aldehyde dehydrogenase 3 family, member A2                                      |
| 499 | 202085_at   | NM_004817 | TJP2       | tight junction protein 2  |
| 500 | 202193_s_at | NM_001144 | AMFR       | autocrine motility factor receptor  |
| 501 | 202196_s_at | NM_013253 | DKK3       | dickkopf (Xenopus laevis) homolog 3   |
| 502 | 202242_at   | NM_004615 | TM4SF2     | transmembrane 4 superfamily mem 2   |
| 503 | 202267_at   | NM_005562 | LAMC2      | laminin, gamma 2, transcript variant 1  |

|     |             |           |             |   |
|-----|-------------|-----------|-------------|---|
| 504 | 202286_s_at | J04152    | GA733-1     | gastrointestinal tumor-assoc antigen                                |
| 505 | 202289_s_at | NM_006997 | TACC2       | transforming, acidic coiled-coil containing protein 2               |
| 506 | 202350_s_at | NM_002380 | MATN2       | matrilin 2 precursor, transcript var 1                              |
| 507 | 202387_at   | NM_004323 | BAG1        | BCL2-associated athanogene  |
| 508 | 202489_s_at | BC005238  |             | FXYD domain-containing ion transport regulator 3                    |
| 509 | 202525_at   | NM_002773 | PRSS8       | protease, serine, 8 (prostasin)                                     |
| 510 | 202552_s_at | NM_016441 | CRIM1       | cysteine-rich motor neuron 1  |
| 511 | 202565_s_at | NM_003174 | SVIL        | supervillin transcript variant 1                                    |
| 512 | 202575_at   | NM_001878 | CRABP2      | cellular retinoic acid-binding protein 2                            |
| 513 | 202597_at   | AU144284  |             | interferon regulatory factor 6                                      |
| 514 | 202668_at   | BF001670  |             | ephrin-B2   |
| 515 | 202712_s_at | NM_020990 | CKMT1       | creatine kinase, mitochondrial 1 nuclear gene mitochondrial protein |
| 516 | 202746_at   | AL021786  |             | PAC 696H22 on chrom Xq21.1-21.2                                     |
| 517 | 202826_at   | NM_003710 | SPINT1      | serine protease inhibitor, Kunitz t 1                               |
| 518 | 202890_at   | T62571    |             | microtubule-associated protein 7                                    |
| 519 | 202936_s_at | NM_000346 | SOX9        | SRY-box 9   |
| 520 | 202994_s_at | Z95331    |             | clone CTA-941F9 on chrom 22q13                                      |
| 521 | 203037_s_at | NM_014751 | KIAA0429    | KIAA0429 gene product   |
| 522 | 203074_at   | NM_001630 | ANXA8       | annexin A8  |
| 523 | 203081_at   | NM_020248 | LOC56998    | beta-catenin-interacting protein ICAT                               |
| 524 | 203126_at   | NM_014214 | IMPA2       | inositol(myo)-1(or 4)-monophosphatase 2                             |
| 525 | 203178_at   | NM_001482 | GATM        | glycine amidinotransferase  |
| 526 | 203240_at   | NM_003890 | FC(gamma)BP | IgG Fc binding protein  |
| 527 | 203327_at   | N22903    |             | insulin-degrading enzyme  |
| 528 | 203355_s_at | NM_015310 | KIAA0942    | KIAA0942 protein  |
| 529 | 203407_at   | NM_002705 | PPL         | Periplakin  |
| 530 | 203408_s_at | NM_002971 | SATB1       | special AT-rich sequence binding protein 1                          |
| 531 | 203430_at   | NM_014320 | SOUL        | putative heme-binding protein                                       |
| 532 | 203453_at   | NM_001038 | SCNN1A      | Na channel, nonvoltage-gated 1 alpha                                |
| 533 | 203485_at   | NM_021136 | RTN1        | reticulon 1   |
| 534 | 203549_s_at | NM_000237 | LPL         | lipoprotein lipase  |
| 535 | 203571_s_at | NM_006829 | APM2        | adipose specific 2  |
| 536 | 203585_at   | NM_007150 | ZNF185      | zinc finger protein 185 (LIM domain)                                |
| 537 | 203636_at   | BE967532  | MID1        | midline 1 (OpitzBBB syndrome)                                       |
| 538 | 203637_s_at | NM_000381 | MID1        |   |
| 539 | 203638_s_at | NM_022969 | FGFR2       | FGF receptor 2 transcript var 2                                     |
| 540 | 203678_at   | NM_014967 | KIAA1018    | KIAA1018 protein  |
| 541 | 203687_at   | NM_002996 | SCYD1       | small inducible cytokine subfam D (Cys-X3-Cys) mem 1                |
| 542 | 203726_s_at | NM_000227 | LAMA3       | laminin, alpha 3  |
| 543 | 203786_s_at | NM_003287 | TPD52L1     | tumor protein D52-like 1  |
| 544 | 203797_at   | AF039555  | VSNL1       | visinin-like protein 1  |
| 545 | 203799_at   | NM_014880 | KIAA0022    | KIAA0022 gene product   |
| 546 | 203812_at   | AB011538  |             | MEGF5   |
| 547 | 203881_s_at | NM_004010 |             | dystrophin transcript variant Dp427p2                               |
| 548 | 203910_at   | NM_004815 | PARG1       | PTPL1-associated RhoGAP 1   |
| 549 | 203917_at   | NM_001338 | CXADR       | coxsackie virus and adenovirus receptor                             |
| 550 | 203961_at   | AL157398  | NEBL        | nebullette protein  |

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| 551 | 203962_s_at | NM_006393 | NEBL          |  |
| 552 | 203963_at   | NM_001218 | CA12          | carbonic anhydrase XII   |
| 553 | 203992_s_at | AF000992  | UTX           | ubiquitous TPR motif, X isoform alternative transcript 1                         |
| 554 | 203997_at   | NM_002829 | PTPN3         | protein tyrosine phosphatase, non-receptor type 3                                |
| 555 | 204005_s_at | NM_002583 | PAWR          | PRKC, apoptosis, WT1, regulator  |
| 556 | 204019_s_at | NM_015677 |               | hypothetical protein DKFZP586F1318   |
| 557 | 204036_at   | AW269335  |               | endothelial differentiation, lysophosphatidic acid G-protein-coupled receptor, 2 |
| 558 | 204037_at   | AW269335  |               | endothelial differentiation, lysophosphatidic acid G-protein-coupled receptor, 2 |
| 559 | 204042_at   | AB020707  |               | KIAA0900 protein   |
| 560 | 204058_at   | AL049699  |               | clone 747H23 on chrom 6q13-15  |
| 561 | 204059_s_at | NM_002395 | ME1           | malic enzyme 1, NADP(+)-dependent, cytosolic                                     |
| 562 | 204072_s_at | NM_023037 | 13CDNA73      | putative gene product  |
| 563 | 204112_s_at | NM_006895 | HNMT          | histamine N-methyltransferase  |
| 564 | 204135_at   | NM_014890 | DOC1          | downregulated in ovarian cancer 1  |
| 565 | 204136_at   | NM_000094 | COL7A1        | collagen, type VII, alpha 1  |
| 566 | 204151_x_at | NM_001353 | AKR1C1        | aldo-keto reductase family 1, mem C1   |
| 567 | 204154_at   | NM_001801 | CDO1          | cysteine dioxygenase, type I   |
| 568 | 204168_at   | NM_002413 | MGST2         | microsomal glutathione S-transferase 2   |
| 569 | 204201_s_at | NM_006264 | PTPN13        | protein tyrosine phosphatase, non-receptor type 13                               |
| 570 | 204204_at   | NM_001860 | SLC31A2       | solute carrier family 31 member 2  |
| 571 | 204224_s_at | NM_000161 | GCH1          | GTP cyclohydrolase 1   |
| 572 | 204254_s_at | NM_000376 | VDR           | vitamin D receptor   |
| 573 | 204345_at   | NM_001856 | COL16A1       | collagen, type XVI, alpha 1  |
| 574 | 204351_at   | NM_005980 | S100P         | S100 calcium-binding protein P   |
| 575 | 204359_at   | NM_013231 | FLRT2         | fibronectin leucine rich transmembrane protein 2                                 |
| 576 | 204363_at   | NM_001993 | F3            | coagulation factor III   |
| 577 | 204379_s_at | NM_000142 | FGFR3         | fibroblast growth factor receptor 3  |
| 578 | 204388_s_at | NM_000240 | MAOA          | monoamine oxidase A  |
| 579 | 204389_at   | NM_000240 | MAOA          | monoamine oxidase A  |
| 580 | 204400_at   | NM_005864 | EFS2          | signal transduction protein  |
| 581 | 204421_s_at | M27968    | FGF           | basic fibroblast growth factor   |
| 582 | 204422_s_at | NM_002006 | FGF2          | fibroblast growth factor 2 (basic)   |
| 583 | 204424_s_at | AL050152  |               | neuronal specific transcription factor DAT1                                      |
| 584 | 204455_at   | NM_001723 | BPAG1         | bullous pemphigoid antigen 1   |
| 585 | 204503_at   | NM_001988 | EVPL          | envoplakin   |
| 586 | 204517_at   | BE962749  | cyclophilin C | peptidylprolyl isomerase C   |
| 587 | 204519_s_at | NM_015993 | LOC51090      | plasmolipin  |
| 588 | 204537_s_at | NM_004961 | GABRE         | gamma-aminobutyric acid A receptor, epsilon transcript variant 1                 |
| 589 | 204591_at   | NM_006614 | CHL1          | cell adhesion molecule with homology to L1CAM                                    |
| 590 | 204600_at   | NM_004443 | EPHB3         | EphB3  |
| 591 | 204671_s_at | BE677131  |               | KIAA0957 protein   |
| 592 | 204675_at   | NM_001047 | SRD5A1        | steroid-5-alpha-reductase, alpha polypeptide 1                                   |

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| 593 | 204718_at   | NM_004445 | EPHB6       | EphB6  |
| 594 | 204719_at   | NM_007168 | ABCA8       | ATP-binding cassette, sub-fam A mem 8                        |
| 595 | 204734_at   | NM_002275 | KRT15       | keratin 15   |
| 596 | 204749_at   | NM_004538 | NAP1L3      | nucleosome assembly protein 1-like 3                         |
| 597 | 204753_s_at | AI810712  |             | hepatic leukemia factor                                      |
| 598 | 204754_at   | AI810712  |             | hepatic leukemia factor                                      |
| 599 | 204755_x_at | M95585    | HLF         | leukemia factor  |
| 600 | 204765_at   | NM_005435 | ARHGEF5     | Rho guanine nucleotide exchange factor 5                     |
| 601 | 204773_at   | NM_004512 | IL11RA      | interleukin 11 receptor, alpha                               |
| 602 | 204773_at   | NM_004512 | IL11RA      |  |
| 603 | 204855_at   | NM_002639 | SERPINB5    | serine (or cysteine) proteinase inhibitor, clade B, member 5 |
| 604 | 204872_at   | NM_007005 | BCE-1       | BCE-1 protein  |
| 605 | 204937_s_at | NM_016325 | ZNF274      | zinc finger protein 274                                      |
| 606 | 204942_s_at | NM_000695 | ALDH3B2     | aldehyde dehydrogenase 3 fam mem B2                          |
| 607 | 204952_at   | NM_014400 | C4.4A       | GPI-anchored metastasis-associated protein homolog           |
| 608 | 204971_at   | NM_005213 | CSTA        | cystatin A (stefin A)  |
| 609 | 204975_at   | NM_001424 | EMP2        | epithelial membrane protein 2                                |
| 610 | 204990_s_at | NM_000213 | ITGB4       | integrin, beta 4   |
| 611 | 205014_at   | NM_005130 | HBP17       | heparin-binding growth factor binding                        |
| 612 | 205019_s_at | NM_004624 | VIPR1       | vasoactive intestinal pep receptor 1                         |
| 613 | 205081_at   | NM_001311 | CRIP1       | cysteine-rich protein 1 (intestinal)                         |
| 614 | 205109_s_at | NM_015320 | ARHGEF4     | Rho guanine nucleotide exchange factor (GEF) 4               |
| 615 | 205128_x_at | NM_000962 | PTGS1       | prostaglandin-endoperoxide synthase 1                        |
| 616 | 205185_at   | NM_006846 | SPINK5      | serine protease inhibitor, Kazal t, 5                        |
| 617 | 205200_at   | NM_003278 | TNA         | tetranectin  |
| 618 | 205206_at   | NM_000216 | KAL1        | Kallmann syndrome 1 sequence                                 |
| 619 | 205236_x_at | NM_003102 | SOD3        | superoxide dismutase 3, extracellular                        |
| 620 | 205251_at   | NM_022817 | PER2        | period homolog 2 transcript variant 1                        |
| 621 | 205259_at   | NM_000901 | NR3C2       | nuclear receptor subfamily 3, group C, member 2              |
| 622 | 205286_at   | U85658    |             | transcription factor ERF-1                                   |
| 623 | 205349_at   | NM_002068 | GNA15       | guanine nucleotide binding protein, alpha 15                 |
| 624 | 205363_at   | NM_003986 | BBOX1       | butyrobetaine (gamma), 2-oxoglutarate dioxygenase 1          |
| 625 | 205382_s_at | NM_001928 | DF          | D component of complement (adipsin)                          |
| 626 | 205384_at   | NM_005031 | FXYD1       | FXYD domain-containing ion transport regulator 1 variant a   |
| 627 | 205403_at   | NM_004633 | IL1R2       | interleukin 1 receptor, type II                              |
| 628 | 205404_at   | NM_005525 | HSD11B1     | hydroxysteroid dehydrogenase 1                               |
| 629 | 205407_at   | NM_021111 | RECK        | reversion-inducing-cysteine-rich protein with kazal motifs   |
| 630 | 205440_s_at | NM_000909 | NPY1R       | neuropeptide Y receptor Y1                                   |
| 631 | 205455_at   | NM_002447 | MST1R       | macrophage stimulating 1 receptor                            |
| 632 | 205464_at   | NM_000336 | SCNN1B      | Na channel, nonvoltage-gated 1, beta                         |
| 633 | 205470_s_at | NM_006853 | KLK11       | kallikrein 11  |
| 634 | 205490_x_at | BF060667  | connexin 31 | gap junction protein, beta 3, 31kD                           |
| 635 | 205498_at   | NM_000163 | GHR         | growth hormone receptor                                      |
| 636 | 205559_s_at | NM_006200 | PCSK5       | proprotein convertase subtilisin/kexin                       |

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|-----|-------------|-----------|----------|--|
|     |             |           |          | type 5   |
| 637 | 205560_at   | NM_006200 | PCSK5    |  |
| 638 | 205569_at   | NM_014398 | TSC403   | similar to lysosome-associated membrane glycoprotein   |
| 639 | 205613_at   | NM_016524 | LOC51760 | BK protein   |
| 640 | 205668_at   | NM_002349 | LY75     | lymphocyte antigen 75                                  |
| 641 | 205672_at   | NM_000380 | XPA      | xeroderma pigmentosum, complementation group A         |
| 642 | 205709_s_at | NM_001263 | CDS1     | CDP-diacylglycerol synthase 1                          |
| 643 | 205730_s_at | NM_014945 | KIAA0843 | KIAA0843 protein                                       |
| 644 | 205765_at   | NM_000777 | CYP3A5   | cyt P450, subfam IIIA, polypep 5                       |
| 645 | 205807_s_at | NM_020127 | TUFT1    | tuftelin 1   |
| 646 | 205857_at   | AI269290  |          | solute carrier family 18, member 2                     |
| 647 | 205883_at   | NM_006006 | ZNF145   | zinc finger protein 145                                |
| 648 | 205900_at   | NM_006121 | KRT1     | keratin 1  |
| 649 | 205933_at   | NM_015559 | KIAA0437 | KIAA0437 protein                                       |
| 650 | 205977_s_at | NM_005232 | EPHA1    | EphA1  |
| 651 | 206032_at   | AI797281  |          | est:we86g02.x1   |
| 652 | 206033_s_at | NM_001941 | DSC3     | desmocollin 3 transcript variant Dsc3a                 |
| 653 | 206068_s_at | AI367275  |          | acyl-Coenzyme A dehydrogenase, long chain              |
| 654 | 206093_x_at | NM_007116 | TNXA     | tenascin XA  |
| 655 | 206122_at   | NM_006942 | SOX20    | SRY-box 20   |
| 656 | 206149_at   | NM_022097 | LOC63928 | hepatocellular carcinoma antigen gene 520              |
| 657 | 206170_at   | NM_000024 | ADRB2    | adrenergic, beta-2-, receptor, surface                 |
| 658 | 206192_at   | L20815    |          | S protein  |
| 659 | 206201_s_at | NM_005924 | MEOX2    | mesenchyme homeo box 2                                 |
| 660 | 206276_at   | NM_003695 | E48      | lymphocyte antigen 6 comp locus D                      |
| 661 | 206315_at   | NM_004750 | CRLF1    | cytokine receptor-like factor 1                        |
| 662 | 206363_at   | NM_005360 | MAF      | v-maf musculoaponeurotic fibrosarcoma oncogene homolog |
| 663 | 206385_s_at | NM_020987 | ANK3     | ankyrin 3, node of Ranvier, tran var 1                 |
| 664 | 206400_at   | NM_002307 | LGALS7   | lectin, galactoside-binding, soluble, 7                |
| 665 | 206453_s_at | NM_016250 | NDRG2    | N-myc downstream-regulated gene 2                      |
| 666 | 206481_s_at | NM_001290 | LDB2     | LIM domain binding 2                                   |
| 667 | 206482_at   | NM_005975 | PTK6     | PTK6 protein tyrosine kinase 6                         |
| 668 | 206515_at   | NM_000896 | CYP4F3   | cyt P450, subfam IVF, polypeptide 3                    |
| 669 | 206539_s_at | NM_023944 | CYP4F12  | cytochrome P450 isoform 4F12                           |
| 670 | 206581_at   | NM_001717 | BNC      | basonuclin   |
| 671 | 206637_at   | NM_014879 | KIAA0001 | KIAA0001 gene product                                  |
| 672 | 206655_s_at | NM_000407 | GP1BB    | glycoprotein Ib (platelet), $\beta$ polypep            |
| 673 | 206693_at   | NM_000880 | IL7      | interleukin 7  |
| 674 | 206884_s_at | NM_003843 | SCEL     | sciellin   |
| 675 | 207002_s_at | NM_002656 | PLAGL1   | pleiomorphic adenoma gene-like 1                       |
| 676 | 207023_x_at | NM_000421 | KRT10    | keratin 10   |
| 677 | 207076_s_at | NM_000050 | ASS      | argininosuccinate synthetase                           |
| 678 | 207121_s_at | NM_002748 | MAPK6    | mitogen-activated protein kinase 6                     |
| 679 | 207655_s_at | NM_013314 | SLP65    | B cell linker protein                                  |
| 680 | 207720_at   | NM_000427 | LOR      | loricrin   |
| 681 | 207761_s_at | NM_014033 |          | DKFZP586A0522 protein                                  |
| 682 | 207843_x_at | NM_001914 | CYB5     | cytochrome b-5   |
| 683 | 207908_at   | NM_000423 | KRT2A    | keratin 2A   |
| 684 | 207943_x_at | NM_006718 | PLAGL1   | pleiomorphic adenoma gene-like transcript variant 2    |

|     |             |           |               |   |
|-----|-------------|-----------|---------------|---|
| 685 | 207955_at   | NM_006664 | SCYA27        | small inducible cytokine subfamily A (Cys-Cys), member 27 |
| 686 | 207996_s_at | NM_004338 | C18ORF1       | chrom 18 open reading frame 1                             |
| 687 | 208096_s_at | NM_030820 |               | hypothetical protein DKFZp564B052                         |
| 688 | 208146_s_at | NM_031311 | LOC54504      | serine carboxypeptidase vitellogenic-like                 |
| 689 | 208161_s_at | NM_020037 | ABCC3         | ATP-binding cassette sub-fam C mem 3                      |
| 690 | 208190_s_at | NM_015925 | LISCH7        | liver-specific bHLH-Zip transcription factor              |
| 691 | 208228_s_at | M87771    | K-sam-III     | secreted FGF receptor                                     |
| 692 | 208609_s_at | NM_019105 | TNXB          | tenascin XB   |
| 693 | 208614_s_at | M62994    |               | thyroid autoantigen                                       |
| 694 | 208651_x_at | M58664    |               | CD24 signal transducer                                    |
| 695 | 208690_s_at | BC000915  |               | Similar to LIM protein,                                   |
| 696 | 208798_x_at | AF204231  | GM88          | 88-kDa Golgi protein                                      |
| 697 | 209047_at   | AL518391  |               | aquaporin 1   |
| 698 | 209159_s_at | AV724216  |               | NDRG family, member 4                                     |
| 699 | 209160_at   | AB018580  | hluPGFS       | aldo-keto reductase family 1, mem C3                      |
| 700 | 209211_at   | AF132818  | CKLF          | colon Kruppel-like factor                                 |
| 701 | 209212_s_at | AB030824  |               | transcription factor BTEB2                                |
| 702 | 209289_at   | AI700518  |               | nuclear factor IB   |
| 703 | 209290_s_at | BC001283  |               | Similar to nuclear factor IB,                             |
| 704 | 209309_at   | D90427    |               | zinc-alpha2-glycoprotein                                  |
| 705 | 209318_x_at | BG547855  |               | pleiomorphic adenoma gene-like 1                          |
| 706 | 209335_at   | AI281593  |               | decorin   |
| 707 | 209348_s_at | AF055376  | c-maf         | short form transcription factor C-MAF                     |
| 708 | 209351_at   | BC002690  |               | keratin 14  |
| 709 | 209357_at   | AF109161  | MRG1          | p35srj  |
| 710 | 209366_x_at | M22865    |               | cytochrome b5   |
| 711 | 209368_at   | AF233336  | EPHX2         | soluble epoxide hydrolase                                 |
| 712 | 209386_at   | AI346835  |               | transmembrane 4 superfam mem 1                            |
| 713 | 209392_at   | L35594    | autotaxin     | ectonucleotide pyrophosphatasephosphodiesterase 2         |
| 714 | 209465_x_at | AL565812  |               | pleiotrophin  |
| 715 | 209493_at   | AF338650  | AIPC          | PDZ domain-containing protein AIPC                        |
| 716 | 209540_at   | NM_000618 | somatomedin C | insulin-like growth factor 1                              |
| 717 | 209550_at   | U35139    |               | NECDIN related protein                                    |
| 718 | 209558_s_at | AB013384  | HIP1R         | huntingtin interacting protein-1-related                  |
| 719 | 209590_at   | AL157414  |               | clone RP11-560A15 on chrom 20                             |
| 720 | 209602_s_at | AI796169  |               | GATA-binding protein 3                                    |
| 721 | 209603_at   | AI796169  |               | GATA-binding protein 3                                    |
| 722 | 209604_s_at | BC003070  |               | GATA-binding protein 3, clone MGC:2346                    |
| 723 | 209605_at   | D87292    | rhodanese     | thiosulfate sulfurtransferase                             |
| 724 | 209656_s_at | AL136550  |               | DKFZp761J17121  |
| 725 | 209679_s_at | BC003379  |               | hyp protein from clone 643, clone MGC:5115                |
| 726 | 209684_at   | AL136924  |               | DKFZp586G2120   |
| 727 | 209687_at   | U19495    | hIRH          | intercrine-alpha  |
| 728 | 209691_s_at | BC003541  | FLJ10488      | hypothetical protein FLJ10488                             |
| 729 | 209699_x_at | U05598    |               | dihydrodiol dehydrogenase                                 |
| 730 | 209732_at   | NM_005127 |               | Sim to C-type lectin, superfam mem 2                      |
| 731 | 209763_at   | AL049176  |               | clone 141H5 on chrom Xq22.1-23                            |
| 732 | 209771_x_at | AA761181  |               | CD24 antigen  |

|     |             |          |            |  |
|-----|-------------|----------|------------|--|
| 733 | 209863 s at | AF091627 |            | CUSP   |
| 734 | 209866 s at | R50822   |            | KIAA0768 protein   |
| 735 | 209975 at   | AF182276 | CYP2E1     | cytochrome P450-2E1  |
| 736 | 210059 s at | BC000433 |            | mitogen-activated protein kinase 13                              |
| 737 | 210096 at   | J02871   |            | lung cytochrome P450 B1  |
| 738 | 210128 s at | U41070   |            | P2 purinergic receptor   |
| 739 | 210298_x at | AF098518 | FHL1       | 4 and 1/2 LIM domains 1 protein isoform B                        |
| 740 | 210347 s at | AF080216 |            | C2H2-type zinc-finger protein                                    |
| 741 | 210372 s at | AF208012 | TPD52L2    | tumor protein D52-like 2   |
| 742 | 210397 at   | U73945   |            | beta-defensin-1  |
| 743 | 210619 s at | AF173154 | HYAL1      | hyaluronoglucosaminidase 1 isof 2                                |
| 744 | 210633 x at | M19156   | KRT10      | acidic keratin-10  |
| 745 | 210715 s at | AF027205 | kop        | Kunitz-type protease inhibitor                                   |
| 746 | 210880 s at | AB001467 | Efs2       |  |
| 747 | 210958 s at | BC003646 |            | clone MGC:4693   |
| 748 | 211043 s at | BC006332 | Lcb        | clathrin, light polypeptide                                      |
| 749 | 211105 s at | U80918   | NF-ATcC    | transcription factor   |
| 750 | 211382_s at | AF220152 | TACC2      | transforming, acidic coiled-coil containing protein 2            |
| 751 | 211458 s at | AF180519 |            | GABA-A receptor-associated                                       |
| 752 | 211596 s at | AB050468 |            | membrane glycoprotein LIG-1                                      |
| 753 | 211597 s at | AB059408 | SMAP31-12  |  |
| 754 | 211653 x at | M33376   |            | pseudo-chlordecone reductase                                     |
| 755 | 211712 s at | BC005830 |            | clone MGC:1925   |
| 756 | 211734_s at | BC005912 |            | Fc fragment IgE, high affinity I, rec for alpha polypep          |
| 757 | 211841_s at | U94510   |            | lymphocyte associated receptor of death 9, alternatively spliced |
| 758 | 211986 at   | BG287862 | desmoyokin | AHNAK nucleoprotein  |
| 759 | 212148 at   | BF967998 |            | FLJ12900 fis,  |
| 760 | 212204 at   | AF132733 |            | DKFZP564G2022 protein  |
| 761 | 212242 at   | AL565074 |            | tubulin, alpha 1 (testis specific)                               |
| 762 | 212327 at   | AK027231 |            | FLJ23578 fis, KIAA1102 protein                                   |
| 763 | 212328 at   | AK027231 |            | FLJ23578 fis, KIAA1102 protein                                   |
| 764 | 212390 at   | AB007923 |            | KIAA0477 gene product  |
| 765 | 212538 at   | AL576253 |            | KIAA1058 protein   |
| 766 | 212543 at   | U83115.1 |            | non-lens beta gamma-crystallin like                              |
| 767 | 212589 at   | BG168858 |            | oncogene TC21  |
| 768 | 212593 s at | N92498   |            | FLJ22071 fis, clone HEP11691                                     |
| 769 | 212724 at   | BG054844 |            | ras homolog gene family, member E                                |
| 770 | 212741 at   | AA923354 |            | monoamine oxidase A  |
| 771 | 212823 s at | AU147160 |            | KIAA0599 protein   |
| 772 | 212841_s at | AI692180 |            | PTPRF interacting protein, binding protein 2                     |
| 773 | 212850_s at | AA584297 |            | low density lipoprotein receptor-related protein 4               |
| 774 | 212875 s at | AP001745 |            | chrom 21 open reading frame 25                                   |
| 775 | 212992 at   | AI935123 |            | ESTs   |
| 776 | 213029 at   | AL110126 |            | DKFZp564H1916  |
| 777 | 213032 at   | AL110126 |            | DKFZp564H1916  |
| 778 | 213050 at   | AA594937 |            | KIAA0633 protein   |
| 779 | 213068 at   | AI146848 |            | dermatopontin  |
| 780 | 213071 at   | AI146848 |            | dermatopontin  |

|     |             |           |       |  |
|-----|-------------|-----------|-------|--|
| 781 | 213106_at   | AI769688  |       | 23664 and 23905 mRNA sequence  |
| 782 | 213110_s_at | AW052179  |       | collagen, type IV, alpha 5   |
| 783 | 213122_at   | AI096375  |       | KIAA1750 protein, partial cds  |
| 784 | 213135_at   | U90902    |       | clone 23612  |
| 785 | 213194_at   | BF059159  |       | Hs.301198 roundabout homolog   |
| 786 | 213227_at   | BE879873  |       | progesterone membrane binding  |
| 787 | 213280_at   | AK000478  |       | FLJ20471 fis   |
| 788 | 213285_at   | AV691491  |       | DKFZp564D1462  |
| 789 | 213287_s_at | X14487    |       | acidic (type I) cytokeratin 10   |
| 790 | 213353_at   | BF693921  |       | ATP-binding cassette, sub-family A, member 5   |
| 791 | 213359_at   | W74620    |       | heterogeneous nuclear ribonucleoprotein D  |
| 792 | 213369_at   | AI825832  |       | DKFZp434A132   |
| 793 | 213375_s_at | N80918    |       | Novel gene mapping to chomo 13   |
| 794 | 213397_x_at | AI761728  |       | DnaJ homolog, subfam C, mem 8  |
| 795 | 213451_x_at | BE044614  |       | tenascin XB  |
| 796 | 213456_at   | AI927000  |       | DKFZP564D206   |
| 797 | 213506_at   | BE965369  |       | proteinase activated receptor-2  |
| 798 | 213556_at   | BE673445  |       | chromosome 19, cosmid R28379   |
| 799 | 213618_at   | AB011152  |       | KIAA0580   |
| 800 | 213695_at   | L48516    | PON3  | paraoxonase 3  |
| 801 | 213707_s_at | NM_005221 | DLX5  | distal-less homeo box 5  |
| 802 | 213725_x_at | AI693140  |       | DKFZp586F071   |
| 803 | 213737_x_at | AI620911  |       |  |
| 804 | 213800_at   | X04697    |       | complement factor H 38-kDa N-term  |
| 805 | 213817_at   | AL049435  |       | DKFZp586B0220  |
| 806 | 213820_s_at | T54159    |       | hypothetical protein MGC10327  |
| 807 | 213844_at   | NM_019102 | HOXA5 | homeo box A5   |
| 808 | 213848_at   | AI655015  |       | DKFZp586F2224  |
| 809 | 213891_s_at | AI927067  |       | FLJ11918 fis   |
| 810 | 213924_at   | BF476502  |       | hypothetical protein FLJ11585  |
| 811 | 213929_at   | AL050204  |       | DKFZp586F1223  |
| 812 | 213933_at   | AW242315  |       | DKFZp586M0723  |
| 813 | 213935_at   | AF007132  |       | clone 23551  |
| 814 | 213942_at   | AL134303  |       | DKFZp547K034_r1  |
| 815 | 213992_at   | AI889941  |       | collagen, type IV, alpha 6   |
| 816 | 213994_s_at | AI885290  |       | spondin 1, extracellular matrix  |
| 817 | 214058_at   | M19720    |       | L-myc protein  |
| 818 | 214132_at   | BG232034  |       | ATP synthase, H <sup>+</sup> transporting, mitochondrial F1 complex, gamma polypeptide 1 |
| 819 | 214164_x_at | BF752277  |       | adaptor-related protein complex 1, gamma 1 subunit                                       |
| 820 | 214234_s_at | X90579    |       | cyp related pseudogene   |
| 821 | 214235_at   | X90579    |       | cyp related pseudogene   |
| 822 | 214247_s_at | AU148057  |       | regulated in glioma  |
| 823 | 214598_at   | AL049977  |       | DKFZp564C122   |
| 824 | 214696_at   | AF070569  |       | clone 24659  |
| 825 | 214753_at   | AW084068  |       | BRCA2 region   |
| 826 | 214823_at   | AF033199  |       | C2H2 zinc finger protein pseudogene  |
| 827 | 215034_s_at | AI189753  |       | FLJ13302 fis   |
| 828 | 215062_at   | AL390143  |       | DKFZp547N074   |
| 829 | 215129_at   | AJ000008  |       | C2 domain containing PI3-kinase  |



|     |             |           |          |   |
|-----|-------------|-----------|----------|---|
| 830 | 215239_x_at | AU132789  |          | zinc finger protein 273   |
| 831 | 215243_s_at | AF099730  | GJB3     | connexin 31   |
| 832 | 215388_s_at | X56210    | FHR-1    | complement Factor H-related 1   |
| 833 | 215513_at   | AF241534  | HYMAI    | hydatidiform mole assoc & imprinted   |
| 834 | 215516_at   | AC005048  |          | BAC clone CTB-15P3 fr 7q22-q31.2  |
| 835 | 215536_at   |           |          | DMA, DMB, HLA-Z1, IPP2, LMP2, TAP1, LMP7, TAP2, DOB, DQB2 and RING8, 9, 13 and 14 genes |
| 836 | 215659_at   | AK025174  |          | FLJ21521  |
| 837 | 215704_at   | AL356504  |          | clone RP1-14N1 chrom 1q21.1-21.3  |
| 838 | 215726_s_at | M22976    |          | cytochrome b5   |
| 839 | 215867_x_at | AL050025  |          | DKFZp564D066  |
| 840 | 216199_s_at | AL109942  |          | clone RP3-473J16 chrom 6q25.3-26  |
| 841 | 216268_s_at | U77914    |          | soluble protein Jagged  |
| 842 | 216333_x_at | M25813    |          | unidentified gene complementary to P450c21  |
| 843 | 216379_x_at | AK000168  |          | CD24 signal transducer  |
| 844 | 216594_x_at | S68290    |          | chlordecone reductase homolog   |
| 845 | 216699_s_at | L10038    |          | pre-pro-protein for kallikrein  |
| 846 | 217087_at   | AF005081  | xp32     | skin-specific protein   |
| 847 | 217528_at   | BF003134  |          | ESTs  |
| 848 | 217707_x_at | AI535683  |          | ESTs  |
| 849 | 217901_at   | BF031829  |          | desmoglein 2  |
| 850 | 217961_at   | NM_017875 | FLJ20551 | hypothetical protein FLJ20551   |
| 851 | 218002_s_at | NM_004887 | SCYB14   | small inducible cytokine subfamily B (Cys-X-Cys), member 14                             |
| 852 | 218170_at   | NM_016048 | LOC51015 | CGI-111 protein   |
| 853 | 218180_s_at | NM_022772 | FLJ21935 | hypothetical protein FLJ21935   |
| 854 | 218186_at   | NM_020387 | CATX-8   | CATX-8 protein  |
| 855 | 218237_s_at | NM_030674 | ATA1     | amino acid transporter system A1  |
| 856 | 218326_s_at | NM_018490 | GPR48    | G protein-coupled receptor 48   |
| 857 | 218434_s_at | NM_023928 |          | hypothetical protein FLJ12389   |
| 858 | 218451_at   | NM_022842 |          | hypothetical protein FLJ22969   |
| 859 | 218499_at   | NM_016542 | LOC51765 | serinethreonine protein kinase MASK   |
| 860 | 218546_at   | NM_024709 |          | hypothetical protein FLJ14146   |
| 861 | 218552_at   | NM_018281 |          | hypothetical protein FLJ10948   |
| 862 | 218603_at   | NM_016217 | LOC51696 | hHDC for homolog of Dros headcase   |
| 863 | 218644_at   | NM_016445 | PLEK2    | pleckstrin 2 (mouse) homolog  |
| 864 | 218651_s_at | NM_018357 |          | hypothetical protein FLJ11196   |
| 865 | 218657_at   | NM_016339 | LOC51195 | Link guanine nucleotide exchange factor II  |
| 866 | 218675_at   | NM_020372 | LOC57100 | organic cation transporter  |
| 867 | 218677_at   | NM_020672 | LOC57402 | S100-type calcium binding protein A14   |
| 868 | 218692_at   | NM_017786 |          | hypothetical protein FLJ20366   |
| 869 | 218704_at   | NM_017763 |          | hypothetical protein FLJ20315   |
| 870 | 218718_at   | NM_016205 | PDGFC    | platelet derived growth factor C  |
| 871 | 218736_s_at | NM_017734 |          | hypothetical protein FLJ20271   |
| 872 | 218751_s_at | NM_018315 |          | hypothetical protein FLJ11071   |
| 873 | 218764_at   | NM_024064 |          | hypothetical protein MGC5363  |
| 874 | 218792_s_at | NM_017688 |          | hypothetical protein FLJ20150   |
| 875 | 218796_at   | NM_017671 |          | hypothetical protein FLJ20116   |
| 876 | 218804_at   | NM_018043 |          | hypothetical protein FLJ10261   |
| 877 | 218806_s_at | AF118887  | VAV-3    | VAV-3 protein   |
| 878 | 218807_at   | NM_006113 | VAV3     | vav 3 oncogene  |


|     |             |           |           |   |
|-----|-------------|-----------|-----------|---|
| 879 | 218816_at   | NM_018214 |           | hypothetical protein FLJ10775                                 |
| 880 | 218820_at   | NM_020215 |           | hypothetical protein DKFZp761F2014                            |
| 881 | 218849_s_at | NM_006663 | RAI       | RelA-associated inhibitor                                     |
| 882 | 218854_at   | NM_013352 | SART-2    | squamous cell carcinoma antigen recog<br>by T cell            |
| 883 | 218901_at   | NM_020353 | LOC57088  | phospholipid scramblase 4                                     |
| 884 | 218919_at   | NM_024699 |           | hypothetical protein FLJ14007                                 |
| 885 | 218963_s_at | NM_015515 |           | DKFZP434G032 protein  |
| 886 | 219010_at   | NM_018265 |           | hypothetical protein FLJ10901                                 |
| 887 | 219054_at   | NM_024563 |           | hypothetical protein FLJ14054                                 |
| 888 | 219064_at   | NM_030569 |           | hypothetical protein MGC10848                                 |
| 889 | 219073_s_at | NM_017784 |           | hypothetical protein FLJ20363                                 |
| 890 | 219090_at   | NM_020689 | NCKX3     | sodium calcium exchanger                                      |
| 891 | 219093_at   | NM_017933 |           | hypothetical protein FLJ20701                                 |
| 892 | 219095_at   | NM_005090 | PLA2G4B   | phospholipase A2, group IVB                                   |
| 893 | 219109_at   | NM_024532 |           | hypothetical protein FLJ22724                                 |
| 894 | 219115_s_at | NM_014432 | IL20RA    | interleukin 20 receptor, alpha                                |
| 895 | 219229_at   | NM_013272 | SLC21A11  | solute carrier family 21, member 11                           |
| 896 | 219232_s_at | NM_022073 |           | hypothetical protein FLJ21620                                 |
| 897 | 219263_at   | NM_024539 |           | hypothetical protein FLJ23516                                 |
| 898 | 219298_at   | NM_024693 |           | hypothetical protein FLJ20909                                 |
| 899 | 219313_at   | NM_017577 |           | hypothetical protein DKFZp434C0328                            |
| 900 | 219368_at   | NM_021963 | NAP1L2    | nucleosome assembly protein 1-like 2                          |
| 901 | 219388_at   | NM_024915 |           | hypothetical protein FLJ13782                                 |
| 902 | 219395_at   | NM_024939 |           | hypothetical protein FLJ21918                                 |
| 903 | 219410_at   | NM_018004 |           | hypothetical protein FLJ10134                                 |
| 904 | 219411_at   | NM_024712 |           | hypothetical protein FLJ13824                                 |
| 905 | 219423_x_at | NM_003790 | TNFRSF12  | TNF receptor superfamily, member 12                           |
| 906 | 219436_s_at | NM_016242 | LOC51705  | endomucin-2   |
| 907 | 219461_at   | AJ236915  |           | pak5 protein  |
| 908 | 219476_at   | NM_024115 |           | hypothetical protein MGC4309                                  |
| 909 | 219489_s_at | NM_017821 |           | hypothetical protein FLJ20435                                 |
| 910 | 219497_s_at | NM_022893 | BCL11A    | B-cell CLL lymphoma 11A                                       |
| 911 | 219518_s_at | NM_025165 |           | hypothetical protein FLJ22637                                 |
| 912 | 219528_s_at | NM_022898 | BCL11B    | B-cell lymphomaleukaemia 11B                                  |
| 913 | 219532_at   | NM_022726 | ELOVL4    | Stargardt disease 3   |
| 914 | 219597_s_at | NM_017434 | DUOX1     | dual oxidase 1  |
| 915 | 219689_at   | NM_020163 | LOC56920  | semaphorin sem2   |
| 916 | 219729_at   | NM_016307 | PRX2      | paired related homeobox protein                               |
| 917 | 219764_at   | NM_007197 | FZD10     | frizzled (Drosophila) homolog 10                              |
| 918 | 219806_s_at | NM_020179 | FN5       | FN5 protein   |
| 919 | 219825_at   | NM_019885 | P450RAI-2 | cyt P450 retinoid metabolizing                                |
| 920 | 219908_at   | NM_014421 | DKK2      | dickkopf homolog 2  |
| 921 | 219936_s_at | NM_023915 | GPR87     | G protein-coupled receptor 87                                 |
| 922 | 219938_s_at | NM_024430 | PSTPIP2   | proline-serine-threonine phosphatase<br>interacting protein 2 |
| 923 | 219970_at   | NM_017655 |           | hypothetical protein FLJ20075                                 |
| 924 | 219976_at   | NM_015888 | HOOK1     | hook1 protein   |
| 925 | 219995_s_at | NM_024702 |           | hypothetical protein FLJ13841                                 |
| 926 | 219998_at   | NM_014181 |           | HSPC159 protein   |
| 927 | 220016_at   | NM_024060 |           | hypothetical protein MGC5395                                  |
| 928 | 220056_at   | NM_021258 | IL22R     | interleukin 22 receptor                                       |
| 929 | 220066_at   | NM_022162 | NOD2      | NOD2 protein  |
| 930 | 220076_at   | NM_019847 | ANKH      | ankylosis, progressive homolog                                |

|     |             |           |          |   |
|-----|-------------|-----------|----------|---|
| 931 | 220161 s at | NM 019114 | EHM2     | EHM2 gene                                 |
| 932 | 220225 at   | NM 016358 | IRX4     | iroquois homeobox protein 4               |
| 933 | 220230 s at | NM 016229 | LOC51700 | cytochrome b5 reductase b5R.2             |
| 934 | 220262 s at | NM 023932 |          | hypothetical protein MGC2487              |
| 935 | 220266 s at | NM 004235 | KLF4     | Kruppel-like factor 4 (gut)               |
| 936 | 220289 s at | NM 017977 |          | hypothetical protein FLJ10040             |
| 937 | 220318 at   | NM 017957 | FLJ20778 | epsin 3                                   |
| 938 | 220413 at   | NM 014579 | ZIP2     | zinc transporter                          |
| 939 | 220414 at   | NM 017422 | CLSP     | calmodulin-like skin protein              |
| 940 | 220428 at   | NM 015717 | LANGERIN | Langerhans cell specific c-type lectin    |
| 941 | 220432 s at | NM 016593 | CYP39A1  | oxysterol 7alpha-hydroxylase              |
| 942 | 220518 at   | NM 024801 |          | hypothetical protein FLJ21551             |
| 943 | 220625 s at | AF115403  |          | Ets transcription factor ESE-2b           |
| 944 | 220723 s at | NM 025087 |          | hypothetical protein FLJ21511             |
| 945 | 220724 at   | NM 025087 |          | hypothetical protein FLJ21511             |
| 946 | 220911 s at | NM 025081 |          | KIAA1305 protein                          |
| 947 | 220945 x at | NM 018050 |          | hypothetical protein FLJ10298             |
| 948 | 221127 s at | NM 006394 | RIG      | regulated in glioma                       |
| 949 | 221215 s at | NM 020639 | ANKRD3   | ankyrin repeat domain 3                   |
| 950 | 221541 at   | AL136861  |          | DKFZp434B044                              |
| 951 | 221667 s at | AF133207  |          | protein kinase H11                        |
| 952 | 221747 at   | AL046979  |          | DKFZp586K0617                             |
| 953 | 221748 s at | AL046979  |          | DKFZp586K0617                             |
| 954 | 221760 at   | BG287153  |          | mannosidase, alpha, class 1A, member 1    |
| 955 | 221796 at   | AA707199  |          | Similar to hyp protein FLJ20093           |
| 956 | 221841 s at | BF514079  |          | Kruppel-like factor 4 (gut)               |
| 957 | 221854 at   | AI378979  |          | ESTs                                      |
| 958 | 221922 at   | AW195581  |          | KIAA0761                                  |
| 959 | 221950 at   | AI478455  |          | empty spiracles homolog 2                 |
| 960 | 222043 at   | AI982754  |          | clusterin                                 |
| 961 | 222102 at   | NM 000847 | GSTA3    | glutathione S-transferase A3              |
| 962 | 222236 s at | AK000253  |          | FLJ20246 fis                              |
| 963 | 222256 s at | AK000550  |          | FLJ20543 fis                              |
| 964 | 222288 at   | AI004009  |          | ESTs                                      |
| 965 | 222290 at   | AA731709  |          | ESTs                                      |
| 966 | 222303 at   | AV700891  |          | ESTs                                      |
| 967 | 266 s at    | L33930    |          | CD24 signal transducer                    |
| 968 | 33322 i at  | X57348    |          | clone 9112                                |
| 969 | 33323 r at  | X57348    |          | clone 9112                                |
| 970 | 35666 at    | U38276    |          | semaphorin III family homolog             |
| 971 | 38340 at    | AB014555  |          | KIAA0655 protein                          |
| 972 | 39248 at    | N74607    |          | za55a01.s1                                |
| 973 | 40016 g at  | AB002301  |          | KIAA0303 gene                             |
| 974 | 40093_at    | X83425    |          | LU gene Lutheran blood group glycoprotein |
| 975 | 40472 at    | AF007155  |          | clone 23763 unknown mRNA                  |
| 976 | 57588 at    | R62432    |          | yg52e11.s1                                |
| 977 | 60474 at    | AA469071  |          | ne17f11.s1                                |
| 978 | 91826 at    | AI219073  |          | qg16e08.x1                                |
| 979 |             |           | PBGD     |   |
| 980 |             |           | MART1    |   |
| 981 |             |           | Me20m    |   |
| 982 |             |           | MAGE-3   |   |
| 983 |             |           |          | Me20m forward primer                      |

|      |  |  |  |                      |
|------|--|--|--|----------------------|
| 984  |  |  |  | Me20m reverse primer |
| 985  |  |  |  | Me20m probe          |
| 986  |  |  |  | PBGD forward primer  |
| 987  |  |  |  | PBGD reverse primer  |
| 988  |  |  |  | PBGD probe           |
| 999  |  |  |  | Tyrosinase           |
| 1000 |  |  |  | Tyrosinase Forward   |
| 1001 |  |  |  | Tyrosinase Reverse   |
| 1002 |  |  |  | Tyrosinase probe     |
| 1003 |  |  |  | MART1 Forward        |
| 1004 |  |  |  | MART1 Reverse        |
| 1005 |  |  |  | MART1 Probe          |
| 1006 |  |  |  | HMB45 Forward        |
| 1007 |  |  |  | gp100 Reverse        |
| 1008 |  |  |  | gp100 Probe          |
| 1009 |  |  |  | PLAB Forward         |
| 1010 |  |  |  | PLAB Reverse         |
| 1011 |  |  |  | PLAB Probe           |

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